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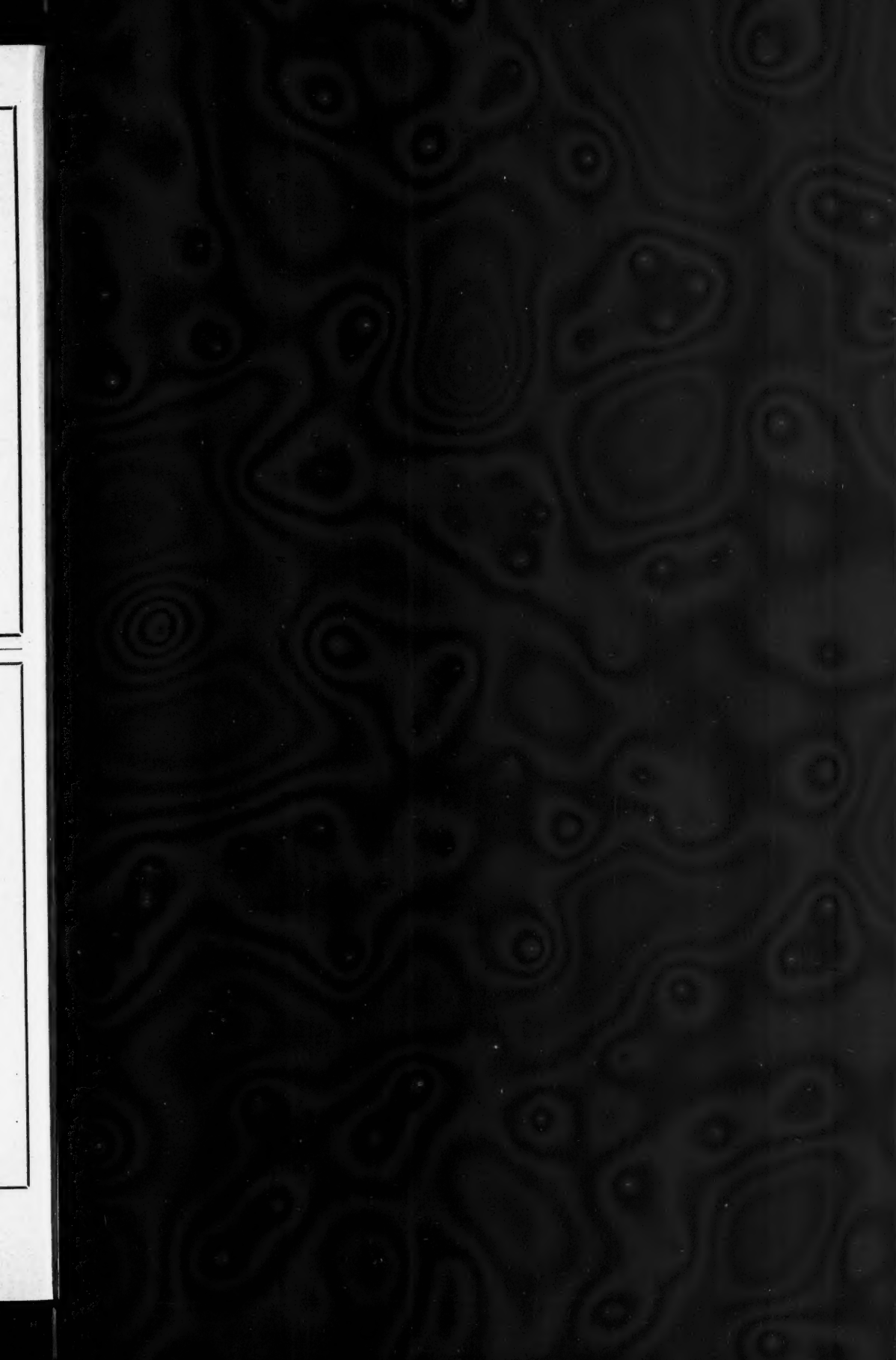
## Contents for May, 1923



# INFLUENCE OF SOIL CONDITION ON BACTERIAL LIFE AND CHANGES IN SOIL SUBSTANCE: II. ABILITY OF SOIL TO BREAK DOWN MANNITE

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In articles already published (1) the author has described preliminary investigations of the ability of soil to break down mannite. These investigations, which were generally qualitative, showed that the ability of the soil to break down this substance, as well as an entire series of other organic substances, depends largely on their chemical conditions.

The close relationship between the mannite-fermentation power of the soil and its content of certain substances (lime and phosphoric acid) made it seem desirable to find a method by which this power might be measured quantitatively. The efforts along that line and the results of a series of special investigations of the requirements for mannite decomposition and its relation to the condition of the soil will be discussed in the present paper.

In hitherto unpublished investigations on the ability of a series of various soils to bind nitrogen from the air, made by mixing the soil portion in question with mannite (2 per cent) and letting them stand for a certain period at a temperature of 25°C., it was observed that much mannite remained in some of the soils. Upon drying some of the soils considerable quantities crystallized on the surface of the soil, while on others only a little crystallization was seen and on others again, none at all. It was evident that the ability of the various soils to break down the mannite added varied greatly. The question now to be answered was how best to determine the amount of unchanged mannite remaining in the soil.

For many reasons direct mannite determination was difficult. On considering the question more carefully it seemed correct to assume that a determination of the entire quantity of soluble organic matter coming from mannite and remaining in the soil would better express the total change of matter and so an attempt was made to determine this content in a way similar to that in which the organic matter in drinking water is usually determined.

The method used in the first step of the investigation was as follows:

An average sample of about 5 gm. was drawn from moist soil containing mannite. This portion was air-dried,[1] weighed and placed in a beaker containing 100 cc. distilled water. After standing for 2 hours with occasional shaking, the mixture was filtered and a certain

[1] Air-drying causes no definite diminution of the content of organic matter.

quantity, as a rule 10 cc., of the clear filtrate was placed in a beaker. To this was added 50 cc. of 0.02 *N* potassium permanganate solution and 3 cc. dilute sulfuric acid (6:100). After heating over a boiling water-bath for 10 minutes, 50 cc. of 0.02 *N* oxalic acid solution was added to the mixture. After continued heating until this was again clear, it was titrated back with 0.02 *N* potassium permanganate solution. The amount of this solution used expresses the amount of soluble organic matter in the soil.

In the course of the investigation this method has been somewhat modified

In the preliminary experiments, the mixture was heated over small water-baths, each containing a single beaker, and as a rule the results of the replicate analyses agreed. However, as the investigations soon became more extensive, the mixture was heated over larger water-baths where there was room for 8 beakers. Now the results of the replicate analyses were often found to disagree. A special investigation showed that this was due to the fact that the temperature attained in the beakers placed in the large water-bath was too low to permit complete oxidation of the organic substances in 10 minutes, and that the temperature varied in the different parts of the water-bath. The degree of heat in the glasses varied between 55–70°C. while in the small water-bath the solution was heated to a temperature of about 80°C. By using a longer period, (20 minutes), and by immersing the beakers directly in the boiling water in the water-bath instead of placing them over the holes the temperature of the solution soon rose to about 80° and the results of the analyses agreed.

The influence of the soil-water content on the power to break down mannite must be established.

In making these investigations a light gray sandy soil and two loamy soils were used. Whereas one portion of sandy soil and one of the loamy soils were unmixed the other portion of sandy soil and the other loamy soil were mixed with carbonate of lime and di-basic potassium phosphate. Tests were made with the application of 4 different amounts of water corresponding to 40, 60, 80 and 100 per cent of the total water-holding capacity.

The water-holding capacity was determined by the following method:

Twenty grams of soil was thoroughly stirred with a surplus of water in a porcelain bowl. The thick soil-gruel was then poured into a funnel tipped with a small moistened filter about 1 cm. long. The funnel was covered with a sheet of glass, and after all dripping had ceased usually on the following day, 5 gm. of the water-soaked soil was weighed into a weighing glass preparatory to determining its oven-dry weight.

In determining the content of water in the air-dry soil, which content must be reckoned with in measuring the water, 5 gm. of soil were weighed out on a watch glass. This, and the water-saturated soil-portions were placed in a desiccator at 100°C. The water-holding capacity is given in per cent of oven-dry soil.

After determining the water-holding capacity, 8 portions of 100 gm. air-dry soil were weighed out into Petri dishes (2 x 10 cm.). This soil was stirred until it formed a homogenous thick layer, loose in structure, at the bottom of each dish. An amount of distilled water was pipetted into each dish corresponding to the desired percentage of the full water-holding capacity of the soil. The water should be dropped—and this is particularly to be recommended in the case of clay soils,—along the edge of the dish, so that it may be absorbed by the soil through capillary action without disturbing its loose structure. In using the largest quantity of water, corresponding to 100 per cent of the water-holding capacity, the loose structure cannot be maintained and clay soils, in particular, tend to become "fluid." This checks the air-supply very materially and as a new factor is thereby introduced into the investigation it becomes impossible by this method to obtain an absolutely true expression for the influence of the water-content on the course of decomposition. The dishes were

covered and kept at a temperature of 25°C. Every fifth day a sample was drawn from each separate dish and the amount of water evaporated during the period replaced by dropping in distilled water.

Results of these investigations may be seen in table 1.

The water-content of the soil determines primarily not only the speed, but also the regularity at which mannite decomposition takes place. The content of water which, under the given conditions, seems most favorable to mannite

TABLE 1

*Influence of the soils content of water on decomposition of mannite*

| MOISTURE IN TERMS OF WATER-HOLDING CAPACITY | CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS* | | | | | |
|---|---|---|---|---|---|---|
| | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days |
| Light gray sandy soil (water-holding capacity 23.5 per cent) | | | | | | |
| *per cent* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* |
| 40 | 32.6 | 28.0 | 29.1 | 30.7 | 31.3 | 24.3 |
| 60 | 33.3 | 31.1 | 31.0 | 24.6 | 17.1 | 13.2 |
| 80 | 32.1 | 29.2 | 29.9 | 20.2 | 9.4 | 3.5 |
| 100 | 28.9 | 21.8 | 18.7 | 15.0 | 12.2 | 11.1 |
| Same soil treated with 0.5 gm. $CaCO_3$ + 0.1 gm. $K_2HPO_4$ | | | | | | |
| 40 | 34.5 | 28.9 | 30.1 | 29.8 | 28.4 | 25.5 |
| 60 | 34.1 | 28.4 | 1.7 | | | |
| 80 | 34.1 | 1.0 | | | | |
| 100 | 32.3 | 7.5 | 0.7 | | | |
| Loamy soil A (water-holding capacity 38.9 per cent) | | | | | | |
| 40 | 23.3 | 26.8 | 26.3 | 25.3 | 17.8 | 10.9 |
| 60 | 23.7 | 24.4 | 20.2 | 10.4 | 1.4 | 0.5 |
| 80 | 24.3 | 20.5 | 14.5 | 3.5 | 0.4 | |
| 100 | 24.7 | 15.0 | 17.0 | 3.1 | 0.5 | |
| Loamy soil B treated with 0.5 gm. $CaCO_3$ + 0.1 gm. $K_2HPO_4$ (water-holding capacity 28.5 per cent) | | | | | | |
| 40 | 31.4 | 21.0 | 17.1 | 10.5 | 1.0 | |
| 60 | 27.8 | 16.6 | 10.0 | 0.8 | | |
| 80 | 29.9 | 1.0 | 0.6 | | | |
| 100 | 26.8 | 1.0 | 0.4 | | | |

* Organic matter is expressed here and in other tables in cubic centimeters of 0.02 *N* potassium permanganate required per 0.5 gm. of dry soil for oxidation. These data are averages of duplicate determinations.

decomposition is that corresponding to about 80 per cent of the absolute water-holding capacity. In the following investigations an amount of water corresponding to 75 per cent of this capacity has been used. This content of water does not destroy the loose structure of the soil whereas soil, and particularly heavy soil, with a water content of 80 per cent tends to become "fluid" and soggy.

Methodical investigations and experience have given us the following complete method for determining the power of mineral soils to break down mannite.

From the soil samples to be tested, an amount of soil corresponding to 100 gm. air-dry soil was weighed out in tumblers 9.5 cm. in diameter and 6.5 cm. deep and mixed with 2 gm. mannite. Distilled water, corresponding to 75 per cent of the water-holding capacity of the soil was added. In investigations of air-dry soils with a somewhat large water-holding capacity, the mannite may be added dissolved in water. When the water has been added, the tumbler must stand undisturbed for 2 to 3 hours so that the water can penetrate the entire soil-portion. The soil is now carefully stirred with the glass spatula and arranged in a homogenous, thick, lightly-resting layer at the bottom of each tumbler. The tumblers were weighed and kept at a temperature of 25°C. The following day the soil was stirred and after 5 days the water lost by evaporation was replaced. When this had thoroughly penetrated the soil, the mass was stirred, (care being taken not to destroy its loose structure) and about 5 gm. were weighed out for air-drying. The tumbler was again weighed and replaced in the incubator. Samples were drawn 5 days later, and then the process was repeated.

The air-dry samples were weighed again, placed in a glass beaker containing 100 cc. of distilled water, left to stand for two hours with occassional shaking, and filtered through filter paper. To 10 cc. of the clear filtrate was added 50 cc. of 0.02 *N* potassium permanganate solution and 3 cc. 6-per cent sulfuric acid.[2] The beaker was then placed in boiling water and allowed to remain for *20 minutes* (the 10 minutes used hitherto was found too short). Fifty cubic centimeters of 0.02 *N* oxalic acid solution was added and when, under constant heating, the liquid became as clear as water (usually, after a few minutes), it was titrated with a 0.02 *N* potassium permanganate solution. The beakers must remain in the water bath until they can be titrated. The amount of potassium permanganate solution used expresses the content of organic matter. The amount of potassium permanganate solution is expressed per ½ gm. of air-dried soil.

## INVESTIGATIONS ON THE ABILITY OF VARIOUS SOILS TO BREAK DOWN MANNITE

The first point to be made clear in the further investigations on this subject was the limit within which the ability of different soils to break down mannite varied.

In these investigations a series of field soils which had been sent in to the laboratory with another object in view were used.

Samples 17, 21, 22, 25, 26, 27, 35, 37, 68 and 69 were drawn from unfertilized plots in local field experiments made in 1916 or 1917 in which the addition of superphosphates caused a greater or less positive reaction. However the lack of phosphoric acid in the soils in question cannot be positively established on the basis of results from this one year. Sample 43 was drawn from plots in a fertilization experiment at Askov Experiment Station which have remained unfertilized for many years, and whose soil is now greatly in need of phosphoric acid (cf. p. 350). Sample 59, from Oelstykke, should also be considered as needing phosphoric acid (cf. p. 351). Samples 10 and 42 are drawn from experimental plots which have been treated with a large quantity of superphosphates for many years, and which cannot be assumed to lack phos-

[2] In order to be certain that the sulfuric acid does not contain organic substances, it is desirable to boil the concentrated acid to be used for a couple of hours.

phoric acid. All the above-named soils are indicated in table 2 by an asterisk (*) in the soil-number column. In the case of the other soils it is not known whether or not they lack phosphoric acid.

Before being used, the soil samples were air-dried and sifted through a 1.5-mm. round-hole sieve. Very fine subdivision of the soil was avoided.

In investigations of this nature, the use of air-dried samples, *particularly when they are used shortly after being dried* can hardly occasion scruples in regard to principle,[3] for it must be remembered that in nature the upper layer of soil is often in that condition, and sometimes for long periods of time. The fact that air-drying, as various scientists have demonstrated, can to some extent change the chemical and microbiological condition of the soil is another matter. Therefore in comparative experiments in decomposition of matter either moist or dry soil should be used in every case.

In order to investigate the extent to which the differences between the power of various soils to decompose mannite depend on chemical or microbiological factors, both *"inoculated" and "uninoculated" cultures have been set aside* in accordance with the author's former principles of investigation (1).

A mannite solution admixed with soil and strongly fermented was used for inoculating. The inoculation was made with a platinum loop, using four loops of liquid culture which were then well mixed into the soil.

The grade of decomposition was determined every five days until 30 days after the experiment was begun, if decomposition had not ceased hitherto. Check determinations of soil samples without admixture of mannite have not been made in every case, but a series of investigations of different soils has shown that the content of water-soluble organic matter per ½-gram air-dry soil, is only by exception greater than the amount which 1–2 cc. of .02 *N* potassium permanganate solution will oxidize. When the content of such organic matter per ½-gram in soils mixed with mannite is within the oxidizing capacity of 3–4 cc. of potassium permanganate solution, decomposition is considered complete.

In table 2, the soil samples are arranged in groups according to their physical condition (sandy soils and loamy soils) and within these groups again according to their reaction. The results show that the ability of soils to break down mannite varied considerably. Soil samples 35, 44, 52, 62, 90, 93 and 95, for instance, showed great power to break down mannite for the mannite in the "inoculated" and "uninoculated" cultures almost entirely disappeared after 5 days. On the other hand, there were many soils which even after 25–30 days contained a large quantity of soluble organic matter. Samples 43 and 61 possessed the least power of decomposing mannite.

Table 2 gives no information in regard to the causes of the differences found. These can not be sought in the varying physical condition of the soils, for examples of slight and of great power to decompose mannite may be found both among light sandy soils and among heavy loamy soils. A closer connection

[3] See second paragraph of footnote 5.

TABLE 2

*The power of various soils to break down mannite*

| SOIL | | GENERAL CONDITION OF THE SOIL | ACID EFFERVESCENCE | REACTION | DEVELOPMENT OF AZOTOBACTER† | PHOSPHORIC ACID CONTENT | | AMMONIUM CHLORIDE SOLUBLE | | CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS % | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Uninoculated | | | | | | Inoculated | | | | | |
| Number | Kind | | | | | $P_2O_5$ soluble in muriatic acid | $P_2O_5$ per liter of $CO_2$-saturated water | CaO | MgO | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days |
| | | | | | | Loamy soils | | | | | | | | | | | | | | | |
| | | | | | | *per cent*‡ | *mgm.* | *per cent*† | *per cent*† | *cc.* % | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* |
| 1 | D. G. U. 3 | h.¶ | none¶ | str. acid¶ | 0 | 0.093 | 0.42 | 0.066 | | 29.7 | 28.6 | 18.6 | 4.8 | 0.7 | | 32.7 | 24.3 | 20.4 | 5.5 | 1.1 | |
| 2 | Stenderup-Eltang | h., poor in humus | none | str. acid | 0 | 0.080 | 0.38 | 0.168 | 0.033 | 38.6 | 36.4 | 35.0 | | | | 36.8 | 27.6 | 14.8 | | | |
| 3 | Aakirkeby | h. | none | acid | 0 | 0.125 | 0.17 | | | 37.1 | 35.6 | 29.4 | 20.2 | 10.3 | 1.0 | 37.4 | 36.5 | 32.6 | 16.9 | 8.7 | 1.1 |
| 4 | Lyngby | | none | acid | 0 | 0.137 | 1.14 | 0.095 | 0.015 | 35.8 | 36.7 | 31.4 | 24.4 | 17.8 | 11.2 | 35.3 | 36.9 | 32.9 | 26.4 | 16.3 | 7.1 |
| 5 | Ny Kirstine-bjerg, Ønslev | h., poor in humus | none | acid | 0 | 0.082 | 0.74 | 0.123 | 0.026 | 35.1 | 14.9 | 5.8 | 2.2§ | | | 34.6 | 17.8 | 5.6 | 1.8§ | | |
| 6 | Rørbæk | r. poor in humus | none | acid | 0 | 0.077 | 0.42 | 0.126 | 0.021 | 40.0 | 38.4 | 41.8 | 35.6 | 34.1 | | 38.0 | 39.0 | 38.7 | 32.2 | 17.7 | |
| 7 | Tanderup | l., rather rich in humus | none | acid | 0 | 0.084 | 0.48 | 0.183 | 0.029 | 37.1 | 37.4 | 27.8 | 5.0§ | | | 37.2 | 35.3 | 27.1 | 8.2§ | 5.0 | |
| 8 | Askov | | none | sl. acid | 0 | 0.066 | 0.30 | 0.160 | 0.025 | 33.8 | 31.2 | 32.1 | 23.4 | 16.5 | 7.9 | 30.9 | 27.7 | 24.8 | 22.7 | 19.0 | 13.7 |
| 9 | Bønlykke, Trustrup | r. h., poor in humus | none | sl. acid | 0 | 0.063 | 0.40 | 0.146 | 0.021 | 40.1 | 37.5 | 35.9 | 30.5 | 27.4 | | 40.7 | 36.8 | 32.1 | 26.9 | 19.5 | |
| 9a | Pederstrup, Assedrup | r. h., poor in humus | none | neut. sl. acid | 0 | 0.065 | 0.58 | 0.240 | 0.030 | 38.0 | 34.6 | 26.2 | 14.9 | 4.8 | | 36.4 | 26.9 | 10.2 | 3.5 | | |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10* | Tystofte | h., poor in humus | none | neut. | 0 | 0.058 | 1.28 | 0.193 | 0.022 | 38.9 | 34.7 | 22.0 | 7.7 | 4.0 | | 38.3 | 4.2 | 3.5 | | | |
| 11 | Hakkebølle-gaard | r. h., poor in humus | none | neut. | 0 | 0.103 | 1.04 | 0.146 | 0.031 | 37.6 | 20.3 | 3.4 | | | | 33.9 | 7.0 | 2.3 | | | |
| 12 | Vrigsted | h. | none | neut. | 0 | 0.082 | 0.54 | 0.330 | 0.035 | 38.6 | 33.5 | 25.6 | 9.3 | 2.7 | | 38.4 | 28.8 | 19.3 | 4.2 | | |
| 13 | Skulsballe, Vrigsted | | none | neut. | 0 | 0.067 | 0.62 | 0.321 | 0.029 | 39.2 | 36.3 | 35.5 | 31.8 | 23.5 | | 39.6 | 36.6 | 34.7 | 30.7 | 20.4 | |
| 14 | Havdrup | l. | none | neut. | 0 | 0.112 | 0.68 | 0.203 | 0.027 | 40.1 | 35.6 | 27.5 | 7.2 | 3.7 | | 39.7 | 36.7 | 25.6 | 5.6 | 3.1 | |
| 15 | Voerladegaard | poor in humus | none | neut. | 0 | 0.134 | 2.22 | 0.218 | 0.027 | 39.5 | 36.6 | 34.4 | 27.0 | 17.0 | | 36.6 | 31.8 | 26.6 | 10.8 | 3.5 | |
| 16 | Gjerlev | | none | neut. | 0 | 0.125 | 0.79 | 0.226 | 0.028 | 38.7 | 35.3 | 24.5 | 19.8 | 6.4 | | 38.0 | 20.9 | 8.5 | 2.1 | | |
| 17* | Ny-Holme-gaard | l., r. poor in humus | v. sl. | neut. | 0–1 | 0.070 | 0.76 | 0.244 | 0.007 | 31.8 | 18.5 | 11.9 | 2.1 | | | 30.3 | 13.9 | 5.5 | 0.8 | 1.2 | |
| 18 | Frammerslev-gaard | l. | none | neut. | 1 | 0.144 | 8.70 | 0.214 | 0.035 | 38.7 | 3.5 | | | | | 4.9 | | | | | |
| 19 | Bellinge | l. | none | neut. | 3 | 0.099 | 1.16 | 0.193 | 0.027 | 34.8 | 26.2 | 23.5 | 15.1 | 2.2 | | 36.8 | 29.8 | 28.7 | 17.6 | 3.1 | |
| 21* | Strøby | h. | none | neut. | 3 | 0.062 | 0.26 | 0.125 | 0.023 | 33.9 | 22.8 | 22.1 | 13.8 | 7.3 | 1.2 | 33.9 | 23.8 | 22.3 | 12.7 | 3.9 | 0.6 |
| 22* | Olstrup | | none | neut. | 3 | 0.081 | 0.64 | 0.197 | | 27.3 | 24.3 | 16.5 | 1.0 | | | 25.9 | 23.8 | 10.0 | 0.6 | | |
| 23 | Bjerager Skov-gaard | r. h., poor in humus | v. sl. | neut. | 3 | 0.081 | 1.14 | 0.280 | 0.035 | 38.4 | 4.0 | | | | | 31.0 | 2.5 | | | | |
| 24 | D. G. U. 6 | | none | neut. | 4 | 0.083 | 1.10 | 0.210 | 0.019 | 38.0 | 28.8 | 28.7 | 22.4 | 15.9 | 10.0 | 34.3 | 32.3 | 27.5 | 22.8 | 17.7 | 9.7 |
| 25* | Forlev 1916 | r. h. | none | neut. | 4 | 0.065 | 0.30 | 0.170 | 0.031 | 34.5 | 22.6 | 16.6 | 8.7 | 5.7 | 1.4 | 37.6 | 19.1 | 20.4 | 10.6 | 4.9 | 1.1 |
| 26* | Forlev 1917 | r. h. | none | neut. | 4 | 0.051 | 0.22 | 0.197 | 0.027 | 39.1 | 25.4 | 22.7 | 24.3 | 19.7 | 13.2 | 38.1 | 24.2 | 22.0 | 20.6 | 15.6 | 6.9 |

* Not included in the summary given in table 6. (See further page 11.)

† The development of azotobacter is expressed in figures within the scale 0–4. 0 indicates no azotobacter development, 4 a maximum development (vigorous, slimy, often folded membrane over the entire surface of the solution).

‡ 100 per cent = weight of air-dry soil.

§ Through an error first determined after 21 days.

⁂ See footnote to table 1. These data are also averages of duplicate determinations.

¶ Abbreviations used are: alk. = alkaline, f. = fine or finely, h. = heavy, l. = light, neut. = neutral, r. = rather, sl. = slightly, str. = strong v = very.

TABLE 2—*Continued*

| SOIL | | GENERAL CONDITION OF THE SOIL | ACID EFFERVESCENCE | REACTION | DEVELOPMENT OF AZOTOBACTER† | PHOSPHORIC ACID CONTENT | | AMMONIUM CHLORIDE SOLUBLE | | CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS ‡ | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Uninoculated | | | | | | Inoculated | | | | | |
| Number | Kind | | | | | $P_2O_5$ soluble in muriatic acid | $P_2O_5$ per liter of $CO_2$-saturated water | CaO | MgO | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days |
| | | | | | | Loamy soils—Continued | | | | | | | | | | | | | | | |
| | | | | | | *per cent*‡ | *mgm.* | *per cent*† | *per cent*† | *cc.* % | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* |
| 27* | Prøvelyst | l., r. rich in humus | none | neut. | 4 | 0.063 | 0.40 | 0.259 | 0.022 | 35.8 | 22.4 | 15.2 | 2.4 | 1.0 | | 36.1 | 21.3 | 14.1 | 4.2 | 1.0 | |
| 28 | Askov, B 4 | l. | none | neut. | 4 | 0.065 | 0.34 | 0.274 | 0.027 | 36.7 | 35.8 | 34.4 | 31.8 | 24.9 | 13.1 | 36.8 | 35.1 | 31.1 | 20.9 | 5.9 | 3.2 |
| 29 | Vium Mølle | l. | none | neut. | 4 | 0.118 | 4.86 | 0.273 | 0.030 | 27.1 | 3.6 | | | | | 3.8 | | | | | |
| 30 | Smaven, Thorsager | l. | sl. | neut. | 4 | 0.071 | 2.66 | 0.207 | 0.018 | 36.8 | 5.6 | | | | | 6.2 | 4.7 | | | | |
| 31 | Oldagergaard | l. | none | neut. | 4 | 0.087 | 1.44 | 0.265 | 0.021 | 31.8 | 9.1 | 2.8 | | | | 2.5 | | | | | |
| 32 | Haarup, Thorsager | l. | v. sl. | neut. | 4 | 0.066 | 2.64 | 0.210 | 0.019 | 38.5 | 30.5 | 5.1 | | | | 11.4 | 3.2 | | | | |
| 33 | Nyskov, Thorsager | v. h. | none | neut. | 4 | 0.114 | 2.38 | 0.390 | 0.055 | 27.1 | 4.7 | | | | | 5.7 | | | | | |
| 34 | Egens, Rønde | h. | none | neut. | 4 | 0.073 | 2.12 | 0.234 | 0.023 | 38.8 | 11.9 | 3.6 | | | | 9.2 | 4.5 | | | | |
| 35* | Lundby | r. rich in humus | none | neut. sl. alk. | 4 | 0.087 | 1.18 | 0.300 | 0.026 | 5.2 | 0.8 | | | | | 0.4 | | | | | |
| 36 | Vejlø, Nakskov | gray, poor in humus | sl. | neut. sl. alk. | 4 | 0.087 | 5.72 | 0.180 | 0.065 | 33.9 | 3.0 | | | | | 2.2 | | | | | |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 37* | Slangerup | | v. sl. | sl. alk. | 4 | 0.102 | 1.58 | 0.285 | 0.017 | 38.6 | 22.2 | 13.0 | 1.5 | | | 38.6 | 16.6 | 7.1 | 0.7 | | |
| 38 | Akurejri, Island a. | rich in iron | sl. | sl. alk. | 4 | 0.212 | 1.58 | 0.601 | 0.148 | 33.5 | 3.6 | | | | | 4.7 | | | | | |
| 39 | Akurejri, Island b. | | none | sl. alk. | 4 | 0.095 | 2.20 | 0.560 | 0.117 | — | — | — | — | — | | 4.1 | | | | | |
| 40 | Brøndsager | v. h. | none | sl. alk. | 4 | 0.080 | 2.70 | 0.282 | 0.027 | 39.4 | 30.0 | 15.0 | 11.6 | | | 27.3 | 5.7 | | | | |
| 42* | Ex-field, Royal Agr. Coll. | h., rich in humus | sl. | alk. | 4 | 0.127 | 3.34 | 0.524 | 0.023 | 19.0 | 5.7 | | | | | 3.8 | | | | | |
| 43* | Askov, B 3 unfertilized 1919 | r. rich in humus | v. sl. | alk. | 4 | 0.046 | 0.26 | 0.356 | 0.014 | 39.8 | 37.6 | 36.7 | 37.5 | 35.7 | 35.4 | 40.4 | 38.1 | 36.8 | 35.9 | 30.3 | 25.3 |
| 44 | Kastrup Mølle | r. rich in humus | str. | alk. | 4 | 0.177 | 10.0 | 0.409 | 0.032 | 1.0 | | | | | | 3.6 | 1.3 | | | | |
| 45 | Horslunde | | r. str. | alk. | 4 | 0.101 | 1.11 | 0.653 | 0.015 | 20.7 | 3.2 | | | | | 3.7 | | | | | |
| 46 | Rønholt | r. poor in humus | sl. | alk. | 4 | 0.093 | 3.28 | 0.343 | 0.020 | 33.1 | 18.6 | 2.1 | | | | 12.6 | 4.3 | | | | |
| 47 | Tystofte | r. h. | r. str. | str. alk. | 4 | 0.062 | 0.88 | — | — | 7.9 | 1.3 | | | | | 2.4 | 0.9 | | | | |
| 48 | Grønvang | r. rich in humus | str. | str. alk. | 4 | 0.056 | 0.54 | 0.450 | 0.026 | 15.7 | 1.7 | | | | | 17.5 | 1.2 | | | | |
| 49 | D. G. U. 8 | h. | str. | str. alk. | 4 | 0.112 | 1.5 | 0.469 | 0.024 | 18.8 | 1.1 | | | | | 11.5 | 0.7 | | | | |
| 50 | Borris | l. | str. | str. alk. | 4 | 0.097 | 0.46 | 0.302 | 0.016 | 36.3 | 7.8 | 2.1 | | | | 29.5 | 1.2 | | | | |
| 51 | Petersdal, Kastrup | l. rich in humus | v. str. | str. alk. | 4 | 0.110 | 6.62 | 0.510 | 0.028 | 32.3 | 17.4 | 5.3 | | | | 22.8 | 3.8 | | | | |
| 52 | Gjerrild | l. | str. | str. alk. | 4 | 0.061 | 3.30 | 0.506 | 0.022 | 3.4 | | | | | | 2.9 | | | | | |
| 53 | Lou, Lolland | r. poor in humus | v. str. | str. alk. | 4 | 0.071 | 0.46 | 1.110 | 0.022 | 37.1 | 32.2 | 23.2 | 11.8 | 4.5 | | 33.7 | 23.6 | 9.9 | 4.2 | | |
| 54 | Baadesgaard, Søllested | h. | sl. | str. alk. | 4 | 0.084 | 2.15 | 0.749 | 0.027 | 17.2 | 3.1 | | | | | 7.7 | 3.2 | | | | |
| 55 | Tjørnehoved, Allerslev | v. h. | sl. | str. alk. | 4 | 0.092 | 2.24 | 0.628 | 0.020 | 32.2 | 5.9 | | | | | 17.8 | 3.4 | | | | |

TABLE 2—*Continued*

| Soil: Number | Soil: Kind | General condition of the soil | Acid effervescence | Reaction | Development of azotobacter† | Phosphoric acid content: $P_2O_5$ soluble in muriatic acid | Phosphoric acid content: $P_2O_5$ per liter of $CO_2$-saturated water | Ammonium chloride soluble: CaO | Ammonium chloride soluble: MgO | Content of organic matter after various decomposition periods ¶: Uninoculated: 5 days | 10 days | 15 days | 20 days | 25 days | 30 days | Inoculated: 5 days | 10 days | 15 days | 10 days | 25 days | 30 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sandy soils** | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | *per cent*‡ | *mgm.* | *per cent*† | *per cent*† | *cc.*¶ | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* |
| 56 | D. G. U. 5 | sandy soil | none | acid | 0 | 0.048 | 0.68 | | | 34.8 | 30.8 | 25.8 | 25.9 | 22.0 | | 36.7 | 29.2 | 29.3 | 25.7 | 23.1 | |
| 57 | Tylstrup | sandy soil, v. f. grained | none | acid | 0 | 0.117 | 1.16 | 0.023 | 0.011 | 36.7 | 37.3 | 34.0 | 22.8 | 9.4 | 2.0 | 36.5 | 38.0 | 35.1 | 24.5 | 9.9 | 2.7 |
| 58 | V. Hassing | sandy soil, v. f. grained | none | acid | 0 | 0.099 | 1.10 | 0.106 | 0.021 | 38.2 | 36.3 | 27.2 | 19.1 | 11.2 | | 35.6 | 27.9 | 14.0 | 4.1 | | |
| 59* | Ølstykke | sandy soil | none | acid | 0 | 0.066 | 0.26 | 0.130 | 0.017 | 38.8 | 36.5 | 35.0 | 30.2 | 13.4 | 4.9 | 38.7 | 36.1 | 34.8 | 30.9 | 19.2 | 8.7 |
| 60 | Lundgaard II | l. sandy soil | none | sl. acid | 0 | 0.050 | 0.44 | 0.056 | 0.014 | 36.0 | 32.0 | 34.0 | 26.3 | 24.6 | | 35.2 | 31.5 | 34.1 | 31.0 | 28.5 | |
| 61 | Askov Sandy field | sandy soil | none | sl. acid | 0 | 0.069 | 1.80 | 0.049 | 0.013 | 36.4 | 36.0 | 33.7 | 33.5 | 32.8 | 32.7 | 37.3 | 37.2 | 33.5 | 33.3 | 34.1 | 30.4 |
| 62 | Faroe Islands | brownish sandy soil, rich in humus | none | sl. acid | 0 | 0.778 | 0.62 | 0.136 | | 8.2 | 1.0 | | | | | 11.3 | 1.6 | | | | |
| 63 | Gørding S. H. | v. l., gray sandy soil | none | sl. acid | 0 | 0.021 | 0.24 | 0.220 | 0.008 | | | | | | | 38.3 | 36.8 | 32.4 | 25.1 | 12.6 | |
| 64 | Haverslev | sandy soil | none | sl. acid | 0 | 0.066 | 0.54 | 0.220 | 0.016 | 40.7 | 39.0 | 34.6 | 28.2 | 15.6 | | 39.5 | 38.5 | 35.4 | 27.6 | 16.6 | |
| 65 | S. P. F. | l., dark gray sandy soil | none | sl. acid | 0 | 0.079 | 1.28 | 0.353 | 0.028 | | | | | | | 36.4 | 21.6 | 9.2 | | | |
| 66 | Hornum | sandy soil | none | sl. acid | 0 | 0.078 | 1.79 | 0.101 | 0.004 | 40.4 | 38.9 | 38.4 | 32.2 | 25.8 | | 40.5 | 38.3 | 36.4 | 25.5 | 11.1 | |
| 67 | Aalsø | sandy soil | none | neut. sl. acid | 0 | 0.094 | 1.18 | | | 39.5 | 39.0 | 35.3 | 16.0 | 1.9 | | 35.1 | 25.4 | 3.4 | | | |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 68 | Grindsted | l., dark gray coarsely grained sandy soil | none | neut. sl. acid | 0 | 0.037 | 0.66 | 0.101 | 0.0 | 36.8 | 30.0 | 22.2 | 17.6 | 10.1 | 5.6 | 28.2 | 30.6 | 20.7 | 16.1 | 19.3 | 17.7 |
| 69* | Fedgaarden | dark gray, rich in humus | none | neut. sl. acid | 0 | 0.017 | 2.26 | 0.166 | | 20.1 | 5.4 | 1.0 | | | | 21.7 | 3.9 | 1.0 | | | |
| 70 | Askov IIII | l. | none | neut. sl. acid | 0 | 0.082 | 2.14 | 0.115 | 0.014 | 37.1 | 36.1 | 36.8 | 34.6 | 32.1 | | 35.8 | 34.7 | 33.1 | 29.1 | 23.5 | |
| 71 | Skrold, Gandrup | l. gray soil | none | neut. sl. acid | 0 | 0.064 | 0.64 | 0.112 | 0.023 | 39.4 | 38.4 | 31.1 | 7.9 | | | 39.5 | 38.4 | 27.9 | 4.9 | | |
| 72 | Gaaser, Gandrup | l. gray | none | neut. | 0 | 0.084 | 2.22 | 0.131 | 0.021 | 38.1 | 35.9 | 13.7 | 3.1 | | | 36.9 | 34.4 | 10.3 | 3.2 | | |
| 73 | Skovsgaard, Hørby | v. f. grained, r. rich in humus | none | neut. | 0 | 0.068 | 1.72 | 0.498 | 0.029 | 40.7 | 38.3 | 32.4 | 22.9 | 4.4 | | 39.5 | 38.9 | 32.6 | 21.9 | 4.1 | |
| 74 | Bregentved | l. | none | neut. | 0 | 0.075 | 1.09 | 0.239 | 0.021 | 38.3 | 35.1 | 29.1 | 5.6 | 1.8 | | 38.0 | 31.1 | 15.2 | 3.2 | | |
| 75 | Thustrup, Skørping | f. grained | none | neut. | 0 | 0.123 | 2.72 | 0.266 | 0.027 | 38.4 | 37.6 | 28.4 | 4.4 | 4.2 | | 36.8 | 22.7 | 4.1 | | | |
| 76 | Enslev, Gjerlev | v. f. grained | none | neut. | 0—1 | 0.094 | 1.84 | 0.292 | 0.018 | 39.1 | 25.8 | 1.6 | | | | 29.4 | 4.3 | | | | |
| 77 | Studsgaard | l. gray | none | neut. | 1 | 0.056 | 0.74 | 0.251 | 0.010 | 38.1 | 38.2 | 37.9 | 36.4 | 31.0 | | 38.2 | 37.6 | 35.9 | 32.6 | 27.1 | |
| 78 | Mejgaard | | none | neut. | 3 | 0.070 | 1.00 | 0.120 | 0.026 | 39.1 | 3.5 | | | | | 5.8 | 3.8 | | | | |
| 79 | Askov Sandmark, G. 2 | | v. sl. | neut. | 3 | 0.082 | 1.16 | 0.202 | 0.008 | 36.7 | 39.6 | 3.0 | | | | 2.9 | | | | | |
| 80 | Elkenøre | v. l. r. coarsely grained, sl. decomposed | none | neut. | 4 | 0.048 | 1.80 | 0.223 | 0.021 | 37.5 | 32.9 | 21.1 | 11.4 | | | 35.7 | 26.8 | 17.1 | 7.2 | | |
| 81 | Toftegaard, Gaaser | l. gray | v. sl. | neut. | 4 | 0.098 | 4.34 | 0.308 | 0.042 | 38.5 | 3.8 | | | | | 2.3 | | | | | |
| 82 | Thorsø, Grenaa | l. | none | neut. | 4 | 0.072 | 2.94 | 0.188 | 0.018 | 38.9 | 35.5 | 33.4 | 17.3 | 3.9 | | 37.1 | 36.5 | 32.0 | 17.0 | 8.0 | |
| 83 | Hagestedgd., Maarsø | v. f., v. poor in humus | none | neut. | 4 | 0.070 | 1.04 | 0.236 | 0.022 | 38.6 | 36.7 | 34.3 | 27.4 | 8.4 | | 37.6 | 35.0 | 24.0 | 7.3 | 4.2 | |
| 84 | Skindbjerg II | l. | v. sl. | neut. sl. alk. | 3 | 0.085 | 3.22 | 0.231 | 0.022 | 13.2 | 4.0 | | | | | 4.8 | 3.5 | | | | |

TABLE 2—*Concluded*

| SOIL | | GENERAL CONDITION OF THE SOIL | ACID EFFERVESCENCE | REACTION | DEVELOPMENT OF AZOTOBACTER† | PHOSPHORIC ACID CONTENT | | AMMOMIUM CHLORIDE SOLUBLE | | CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS # | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Uninoculated | | | | | | Inoculated | | | | | |
| Number | Kind | | | | | $P_2O_5$ soluble in muriatic acid | $P_2O_5$ per liter of $CO_2$-saturated water | CaO | MgO | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days |
| | | | | | | Sandy soils—Continued | | | | | | | | | | | | | | | |
| | | | | | | *per cent*‡ | *mgm.* | *per cent*† | *per cent*† | *cc.* % | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* |
| 85 | Skindbjerg I | | none | neut. sl. alk. | 4 | 0.064 | 0.92 | 0.219 | 0.018 | 34.5 | 11.0 | 3.2 | | | | 7.1 | 3.7 | | | | |
| 86 | Enslevgaard, Grenaa | r. rich in humus | str. | sl. alk. | 4 | 0.095 | 4.48 | 0.924 | 0.025 | 17.9 | 3.5 | | | | | 6.8 | | | | | |
| 87 | Fladsaagaard, Næstved | l., r. coarse | sl. | sl. alk. | 4 | 0.150 | 2.42 | 0.729 | 0.023 | 19.4 | 3.5 | | | | | 3.1 | | | | | |
| 88 | Aastrup | rich in humus | r. str. | alk. | 4 | 0.095 | 2.34 | 0.646 | 0.029 | 4.9 | 2.6 | | | | | 4.9 | 2.2 | | | | |
| 89 | Petersdal, Kastrup | coarse, rich in humus | str. | alk. | 4 | 0.143 | 12.2 | 0.521 | 0.029 | 33.3 | 13.9 | 5.2 | | | | 21.4 | 3.1 | | | | |
| 90 | Gjerrild | r. rich in humus | str. | alk. | 4 | 0.114 | 8.46 | 0.500 | 0.020 | 3.4 | | | | | | 2.9 | | | | | |
| 91 | Teglværksgd., Jyderup | l., poor in humus | r. str. | alk. | 4 | 0.081 | 1.61 | 0.332 | 0.013 | 28.8 | 3.2 | | | | | 6.9 | | | | | |
| 92 | Mørkøv | v. l., coarse | r. str. | alk. | 4 | 0.075 | 1.75 | 0.435 | 0.012 | 9.0 | 5.7 | | | | | 3.0 | | | | | |
| 93 | Trustrup brick and lime works | | v. str. | str. alk. | 4 | 0.083 | 2.54 | 0.567 | 0.018 | 2.9 | | | | | | 2.9 | | | | | |
| 94 | Hulemosegd., Nyraad | v. f. grained, poor in humus | str. | str. alk. | 4 | 0.054 | 1.55 | 0.406 | 0.016 | 36.0 | 30.6 | 22.3 | 5.3 | | | 30.9 | 12.0 | 3.1 | | | |
| 95 | Thisted field | r. rich in humus | r. str. | str. alk. | 4 | 0.110 | 2.74 | 0.693 | 0.025 | 6.1 | | | | | | 5.4 | | | | | |

exists between the reaction of the soil, particularly its content of buffers, measured by its azotobacter development in the azotobacter test, and its power of decomposition. *The non-lime-requiring soils* with azotobacter development possess, as a rule, a far greater power to decompose mannite than the lime-requiring soils without azotobacter development. The author found this in his earlier investigations (1) on the decomposition of peptone and cellulose. Many exceptions, however, are found which show that the relation depends to a large extent on some factor other than lime-requirement. If we confine ourselves solely to the strongly acid soils, we observe that these, with but a single exception (table 2), possess a comparatively slight power to decompose mannite.

Earlier investigations of the author (1) on the influence of the condition of the soil on the decomposition of various organic substances showed that the content of phosphoric acid in a form available for the microörganisms in question was particularly effective in determining the speed of decomposition. The influence of this factor in this investigation was noted by determining, in the case of most of the soil samples, the content of phosphoric acid present in combinations soluble in boiling 20 per cent muriatic acid and in carbonic acid.[4] Determinations were likewise made of the lime- and magnesia-content of the soil by a method previously described by the author (2).

No connection can be shown between the content of phosphoric acid in muriatic-acid-soluble combinations and the speed of mannite decomposition. However, the results (disregarding the group of non-basic soils) in which the conditions for mannite decomposition, as mentioned above, are poor, indicate definitely that those soils showing the greatest saturation-concentration of $P_2O_5$ in carbonic-acid-saturated water possessed as a rule the greatest power to break down mannite.

Non-lime requiring inoculated soils which had given off more than 1.2 mgm. $P_2O_5$ (per liter of $CO_2$-saturated water), decomposed practically all the mannite applied within 10 days. On the other hand, soils which had not given off more than 0.5 mgm. of $P_2O_5$ possessed a very small power of mannite decomposition. No absolute relation can be shown between the saturation-concentration of $P_2O_5$ and the speed of decomposition within the limits of these values. In the case of soils with a pronounced alkaline reaction, the above-named values seem to lie within much narrower limits, for in every case in which the saturation-concentration exceeds 0.5 mgm. $P_2O_5$ per liter, a rapid mannite decomposition has taken place in the inoculated cultures.

A particularly striking example of the influence of phosphoric acid on the speed of mannite decomposition may be seen by considering the results obtained in using soil 43, described on page 350. It is the only one of the strongly alkaline-

[4] The method used by Mitscherlich (5) in determining the saturation-concentration of $P_2O_5$ in carbonic-acid-saturated water as the expression of the solubility of the phosphoric acid in soil, is used with small modifications. The process of decomposition takes place at a temperature of 30°C.

reacting soils which caused a very slow mannite decomposition. The saturation-concentration of $P_2O_5$ in carbonic-acid-extract was extremely small, (0.26 mgm. per liter).[5] We observe, too, that several of the non-lime-requiring soils, even though they had a small saturation-concentration of $P_2O_5$ in carbonic-acid-extract, caused rapid mannite decomposition.

It was observed that in some of the soils 3–6 days after the decomposition experiment had begun, slimy azotobacter coatings appeared. A closer inspection, as a rule, showed that the entire soil portion was filled with these coatings, often rendering it sticky. This would seem to indicate a rather large content of easily soluble, phosphoric-acid combinations (table 3).

It is possible that some other soils tested showed the azotobacter coating described, but they were not examined systematically. In the investigations

TABLE 3

| SOIL NUMBER | LITMUS REACTION | $CaCO_3$ SOLUBLE IN AMMONIUM CHLORIDE | $P_2O_5$ IN COMBINATIONS SOLUBLE IN MURIATIC ACID | SATURATION-CONCENTRATION OF $P_2O_5$ IN CARBONIC ACID EXTRACT | TIME FOR COMPLETE DECOMPOSITION IN INOCULATED CULTURES |
|---|---|---|---|---|---|
| | | *per cent* | | *mgm. per liter* | *days* |
| 18 (table 3) | Neutral | 0.21 | 0.144 | 8.70 | 5 |
| 29 (table 3) | Neutral | 0.27 | 0.118 | 4.86 | 5 |
| 31 (table 3) | Neutral | 0.27 | 0.087 | 1.44 | 5 |
| 33 (table 3) | Neutral | 0.39 | 0.114 | 2.38 | 5 |
| 44 (table 3) | Alkaline | 0.41 | 0.177 | 10.00 | 5 |
| 9 (table 5) | Alkaline | 0.58 | 0.137 | 1.10 | 5 |
| 10 (table 5) | Alkaline | 0.52 | 0.123 | 3.34 | 5 |
| a* | Alkaline | 0.37 | 0.062 | 2.16 | 10 |
| b* | Alkaline | 0.26 | 0.112 | 6.52 | 5 |

* These soils belong to another series of investigations and are therefore not quoted in any of the tables of the present paper.

[5] A decomposition experiment with the same soil samples was made about 12 months later. During that time the soil had lain in an air-dry, pulverized condition in a glass jar placed in the store-room of the laboratory. The decomposition experiment was made with "inoculated" cultures. It was observed that mannite decomposition took place much more quickly than the first time. The values expressing mannite decomposition may be compared as follows:

| Year of experiment | Mgm. $P_2O_5$ per liter $CO_2$ saturated water | Organic-matter content after: 5 days | 10 days | 15 days | 20 days | 25 days | 30 days |
|---|---|---|---|---|---|---|---|
| 1919 | 0.26 | 40.4 | 38.1 | 36.8 | 35.9 | 30.3 | 25.3 |
| 1920 | 0.85 | 36.0 | 22.9 | 6.2 | | | |

The solubility of phosphoric acid in the soil was considerably greater in the soil sample that has been stored than in the comparatively fresh one and in relation to the experiments already described it seems reasonable to suppose that this storage was the cause of the more rapid decomposition of mannite. Rahn (6) has shown that air-drying increased this power considerably, and in view of the very important influence, which phosphoric acid exerts on the various biological changes in matter in the soil, it may be considered probable that this increase has been caused by the effect of air-drying on the solubility of the phosphoric acid in the soil. Various investigations (4) indicate also that air-drying of soil increases the solubility of various substances in it.

described later (table 4), with direct addition of a large quantity of phosphates, the azotobacter coating appeared very frequently in the "phosphate" tumblers, but never in those tumblers to which no phosphates were added. Azotobacter coating in soil under conditions present in ordinary decomposition experiments, may, then, plainly enough be considered a qualitative, biological, phosphoric-acid reaction. It is probable, however, that a visible azotobacter coating may require a larger content of these combinations than is necessary to satisfy the need of ordinary crops.

In the case of soils with an acid reaction, and particularly in acid loamy soils, no connection can be found between the variations in the saturation-concentration of $P_2O_5$ in carbonic-acid extracts and the speed of mannite-decomposition. These soils, with a few exceptions among sandy soils, always broke down mannite slowly.

The exceptions were the two sandy soils rich in humus, no. 62 (Faroe Islands) and no. 69 (Fedgaard), which have just as much claim to be considered humus soils as mineral soils. Humus soils seem to show a very different relation to mannite decomposition than mineral soils. They often contain a comparatively large amount of phosphoric-acid combinations soluble in carbonic acid. There will probably be occasion to return later to this problem which must be made the subject of special investigations.

The relation between the soil's content of lime soluble in ammonium chloride and the course of mannite-decomposition does in the main correspond to that described for the reaction of the soil. That a considerable content of the nutritive substance calcium cannot neutralize the restraining effect which the lack of basic substances in the soil exerts on the decomposition of mannite, is often proved in the results. This is particularly apparent in the investigations of soil 73.

It seems impossible to show any relation between the soil's content of magnesia soluble in ammonium chloride (which indeed varies within much narrower limits than the content of lime soluble in ammonium chloride) and its power to break down mannite. This may possibly be explained by the fact that no relation exists between the content of magnesia and soil reaction (cf. table 2.)

As table 2 shows, mannite decomposition as a rule took place somewhat more quickly in the "inoculated" than in the "uninoculated" cultures. In a few of the soils (15, 32, 40 and 67), the difference was quite apparent. This expresses the fact, perhaps, that the change in the microbiological conditions of the soil did not keep pace with the possible changes which took place in the chemical condition. In most cases the difference disappeared at the end of five or ten days, which shows that the majority of the cultivated mineral soils contain a sufficient number of mannite-decomposing organisms to enable it to attain an approximately maximum decomposition-speed, and *that the difference in power of mineral soils to break down mannite is primarily due to differences in their chemical composition.*

TABLE 4

*The influence of various substances on mannite decomposition in the soil (inoculated cultures)*

| KIND OF SOIL AND NUMBER | ACID EFFERVESCENCE | LITMUS REACTION | DEVELOPMENT OF AZOTOBACTER | TREATMENT* | CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS† | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days |
| **Loamy soils** | | | | | | | | | | |
| | | | | | *cc.*† | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* |
| *Forlev (1917)* | none¶ | neut.¶ | 4 | none | 32.3 | 26.9 | 24.4 | 20.8 | 15.1 | |
| (No. 26, Table 2) | | | | $CaCO_3$ | 29.9 | 6.0 | 1.1 | | | |
| r. h. | | | | $CaCO_3 + CaHPO_4$ | 27.4 | 1.0 | | | | |
| | | | | $CaCO_3 + KCl$ | 33.2 | 5.8 | | | | |
| | | | | $CaCO_3 + KCl + CaHPO_4$ | 32.5 | 1.1 | | | | |
| | | | | $CaCO_3 + K_2HPO_4$ | 29.4 | 0.9 | | | | |
| | | | | $K_2HPO_4$ | 28.5 | | 8.1 | 3.8 | | |
| *Strøby* | none | neut. | 3 | none | 27.1 | 28.6 | 27.1 | 14.1 | 2.4 | |
| (No. 21, Table 2) | | | | $CaCO_3$ | 18.1 | 0.7 | | | | |
| h. | | | | $CaCO_3 + CaHPO_4$ | 1.1 | | | | | |
| | | | | $CaCO_3 + KCl$ | 13.0 | 0.5 | | | | |
| | | | | $CaCO_3 + KCl + CaHPO_4$ | 1.1 | | | | | |
| | | | | $CaCO_3 + K_2HPO_4$ | 0.9 | | | | | |
| | | | | $K_2HPO_4$ | 21.2 | 17.6 | 19.7 | 6.9 | 3.7 | |
| *Askov* | none | sl. acid | 0 | none | 34.5 | 29.3 | 27.5 | 27.2 | 24.8 | 15.6 |
| (No. 8, Table 2) | | | | $CaCO_3$ | 33.9 | 30.3 | 1.6 | | | |
| | | | | $CaCO_3 + CaHPO_4$ | 28.8 | 1.2 | | | | |
| | | | | $CaCO_3 + K_2HPO_4$ | 20.2 | 1.6 | | | | |
| | | | | $CaHPO_4$ | 34.8 | 31.6 | 24.4 | 16.6 | 0.9 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $K_2HPO_4$ | 33.3 | 30.8 | 26.1 | 18.3 | 8.2 | 1.4 |
| | | | | $KH_2PO_4$ | 35.5 | ‡ | 30.9 | 20.4 | 10.8 | 1.0 |
| *Askov (B 3)* (No. 43, Table 2) | v. sl. | alk. | 4 | none | 39.5 | 36.1 | 27.3 | 15.6 | 7.5 | |
| | | | | $CaCO_3$ | 39.1 | 36.8 | 24.9 | 11.9 | 3.1 | |
| | | | | $CaCO_3 + CaHPO_4$ | 4.1 | | | | | |
| | | | | $CaCO_3 + KCl$ | 39.1 | 37.4 | 29.3 | 18.9 | 10.0 | |
| | | | | $CaCO_3 + CaHPO_4 + KCl$ | 3.1 | | | | | |
| | | | | $CaCO_3 + CaHPO_4 + KCl + (NH_4)_2SO_4$ | 1.3 | | | | | |
| | | | | $CaHPO_4$ | 2.5 | | | | | |
| | | | | $K_2HPO_4$ | 2.3 | | | | | |
| | | | | $CaCO_3 + K_2HPO_4$ | 2.0 | | | | | |
| | | | | $CaCO_3 + KH_2PO_4$ | 4.1 | | | | | |
| *Slangerup (1917)* (No. 37, Table 2) | v. sl. | sl. alk. | 4 | none | 32.2 | 26.0 | 17.0 | 0.1 | | |
| | | | | $CaCO_3$ | 29.4 | 24.0 | 16.0 | 0.0 | | |
| | | | | $CaCO_3 + CaHPO_4$ | 25.1 | 25.4 | 14.6 | 0.1 | | |
| | | | | $CaCO_3 + CaHPO_4 + KCl$ | 29.8 | 26.6 | 25.0 | 6.3 | 0.2 | |
| | | | | $CaCO_3 + K_2HPO_4$ | 31.7 | 21.7 | 5.3 | 0.1 | | |
| | | | | $K_2HPO_4$ | 31.4 | 21.8 | 5.0 | 0.2 | | |
| *Slangerup (1919)* | v. sl. | sl. alk. | 4 | none | 34.0 | 20.4 | 6.8 | 2.7 | | |
| | | | | $CaCO_3 + CaHPO_4 + K_2SO_4$ | 4.4 | | | | | |
| | | | | $CaCO_3 + CaHPO_4 + K_2SO_4 + (NH_4)_2SO_4$ | 3.3 | | | | | |
| *Lyngby I* l., r. poor in humus | none | acid | 0 | none | 35.6 | 20.5 | 0.3 | | | |
| | | | | $CaCO_3$ | 31.4 | 1.0 | | | | |
| | | | | $CaCO_3 + CaHPO_4$ | 31.8 | 1.0 | | | | |

* Of the various substances the following quantities are used per tumbler (75 gm. soil) 0.75 gm. $CaCO_3$; 0.30 gm. $CaHPO_4$; 0.15 gm. $K_2HPO_4$; 0.113 gm. $KH_2PO_4$; and 0.075 gm. KCl.

† See footnote to table 1. These data are also averages.

‡ As in this determination a serious error has probably been made, the average is not calculated.

¶ Abbreviations used are: alk. = alkaline, h. = heavy, l. = light, neut. = neutral, r. = rather, sl. = slight or slightly, v. = very.

TABLE 4—*Continued*

| KIND OF SOIL AND NUMBER | ACID EFFERVESCENCE | LITMUS REACTION | DEVELOPMENT OF AZOTOBACTER | TREATMENT* | CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS† | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days |
| **Loamy soils—Continued** | | | | | | | | | | |
| | | | | | *cc.*† | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* |
| *Lyngby P.* poor in humus | none | acid | 0 | none | 38.5 | 35.2 | 22.4 | 6.6 | 3.3 | |
| | | | | $CaCO_3$ | 37.4 | 30.7 | 9.2 | 1.5 | | |
| | | | | $CaCO_3 + CaHPO_4$ | 35.8 | 5.1 | 2.1 | | | |
| *Aakirkeby* (No. 3, Table 2) h. | none | acid | 0 | none | 33.8 | 20.3 | 7.6 | | | |
| | | | | $CaCO_3$ | 24.6 | 0.9 | | | | |
| | | | | $CaCO_3 + CaHPO_4$ | 14.6 | 0.8 | | | | |
| *Kildebrønde* h. | none | sl. acid | 0 | none | 38.7 | 32.4 | 23.4 | 16.5 | 9.5 | |
| | | | | $CaCO_3$ | 5.1 | | | | | |
| | | | | $CaCO_3 + CaHPO_4$ | 6.3 | | | | | |
| **Sandy soils** | | | | | | | | | | |
| *Lundgaard II* (No. 60, Table 2) l. | none | sl. acid | 0 | none | 33.9 | 34.4 | 33.1 | 26.8 | 19.6 | 13.4 |
| | | | | $CaCO_3$ | (31.4) | 34.1 | 30.3 | 21.0 | 2.6 | |
| | | | | $CaCO_3 + CaHPO_4$ | 27.7 | 25.1 | 4.7 | 1.7 | | |
| | | | | $CaCO_3 + K_2HPO_4$ | 36.1 | 28.4 | 24.3 | 5.6 | 1.9 | |
| | | | | $CaHPO_4$ | (29.6) | 34.5 | 31.7 | 20.3 | 17.7 | 12.1 |
| | | | | $K_2HPO_4$ | 35.6 | 31.5 | 29.7 | 30.9 | 21.4 | 18.7 |
| | | | | $KH_2PO_4$ | 34.9 | 31.2 | 28.2 | 21.6 | 11.3 | 6.0 |
| *Studsgaard* (No. 77, Table 2)..... l. gray | none | neut. | 1 | none | 38.6 | 37.6 | 33.5 | 26.3 | 17.7 | |
| | | | | $CaCO_3 + CaHPO_4$ | 37.5 | 36.5 | 28.5 | 18.8 | 10.4 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $CaCO_3 + CaHPO_4$ | 5.2 | | | | | |
| | | | | $CaCO_3 + CaHPO_4 + KCl$ | 4.4 | | | | | |
| | | | | $CaCO_3 + CaHPO_4 + KCl + (NH_4)_2SO_4$ | 3.4 | | | | | |
| | | | | $CaHPO_4$ | 5.6 | | | | | |
| | | | | $K_2HPO_4$ | 38.4 | 32.9 | 6.3 | 3.2 | | |
| | | | | $CaCO_3 + K_2HPO_4$ | 4.0 | | | | | |
| | | | | $CaCO_3 + KCl$ | 37.9 | 37.7 | 33.7 | 27.1 | 20.2 | |
| | | | | $CaCO_3 + KH_2PO_4$ | 3.9 | | | | | |
| *Ølstykke* No. 59, Table 2) | none | acid | 0 | none | 38.7 | 36.1 | 34.8 | 30.9 | 19.2 | 8.7 |
| | | | | $CaCO_3$ | 39.3 | 34.0 | 27.5 | 6.5 | 3.6 | |
| *Blaahøj, Grindsted* (No. 20, Table 5) l., dark gray, rich in humus | none | sl. acid | 0 | none | 40.7 | 39.3 | 39.5 | 39.4 | 38.8 | 36.8 |
| | | | | $CaCO_3$ | 40.0 | 36.5 | 32.5 | 29.6 | 24.5 | 14.8 |
| | | | | $CaCO_3 + CaHPO_4$ | 5.4 | | | | | |

Table 4 gives the influence *of chemical factors* on the decomposition of mannite in soils. The principles involved have been described (1). Soil samples which previous investigations had shown to possess a comparatively slight power to break down mannite were used. The method of procedure may be seen in the table. Only "inoculated" cultures were used.[6]

The results of these investigations show that *the soil's content of basic lime and of phosphoric acid combinations determine the speed at which mannite decomposition takes place.* As the author has already shown, 1914 (1) in the report of investigations of the decomposition of cellulose, carbonate of lime may further decomposition in four ways; (1) by acting directly as lime nutrition on the microbes in question; (2) by changing the reaction; (3) by giving acid saturation (buffer effect), and (4) by transforming the plant nutritive substances in the soil that are soluble only with difficulty, into a form readily assimilable by the micro-organisms in question. While the investigations of the requirements necessary for cellulose decomposition in mineral soils showed that the first effect was of but little value for this process which transpired just as quickly when di-basic potassium phosphate ($K_2HPO_4$) was used as when this phosphate was used in connection with carbonate of lime, the results of the present investigations revealed the fact that the calcium-ion itself is of great importance in the work done by the mannite decomposing microbes. The use of di-basic potassium phosphate, in spite of its buffer effect and power to change the reaction toward the alkaline, has repeatedly resulted in a far slower mannite-decomposition than the use of that salt or of calcium phosphate in combination with carbonate of lime. This relation appears very plainly in decomposition experiments using soil with an acid reaction from Lundgaard. Here the use of potassium phosphate alone has had no effect whatsoever on mannite-decomposition. In loamy soil from Askov with acid reaction and the sandy soil from Studsgaard the effect of $K_2HPO_4$ used alone is small in comparison with the effect obtained by using this salt in combination with $CaCO_3$. Di-basic potassium phosphate, moreover, seemed under certain conditionsto exert a restraining influence on mannite-decomposition.

That the power of carbonate of lime to change reaction and cause acid-saturation can, under given conditions, have an important effect on the speed of mannite-decomposition, may be seen very plainly in the investigations of the two lime-requiring soils (acid reaction and no azotobacter development) from Askov and Lundgaard. The addition of carbonate of lime to di-basic phosphate of lime ($CaHPO_4$) to these soils greatly stimulated mannite decomposition. Of the soil from Lundgaard, the portion mixed with calcium phosphate alone

[6] Note must be made of the fact that several of these investigations, those, for instance, with soil from Lundgaard, were made before the methods of analysis was sufficiently developed. This may explain why the values found for the content of organic matter at the close of a period can be greater than in the preceding period, a circumstance not encountered after the improved analysis method was brought into use. However, the results obtained threw sufficient light on the matter in question to warrant their being included here.

did not perceptibly break down mannite more quickly than the soil portion to which nothing had been added. Only when both carbonate of lime and phosphate of lime were added was the process hastened to any appreciable degree.

Whether the power of carbonate of lime to stimulate mannite-decomposition depends on its power to render more active the difficultly soluble phosphoric-acid-combinations in the soil, cannot be answered positively on the basis of the results of these experiments (1, p. 132–34 German translation). It seems probable, however, that the very stimulating effect which carbonate of lime used alone had on mannite-decomposition in soils poor in phosphates soluble in carbonic acid, depends largely on that power.

It is possible also that the power which lime possesses of rendering difficultly soluble phosphoric-acid-combinations more soluble is responsible for the often observed slight stimulation of mannite-decomposition after the addition of easily soluble phosphates as a supplement to lime. On the whole, the results show plainly enough the great influence exerted by the presence of easily soluble phosphoric-acid-combinations on the speed of mannite-decomposition. On the other hand, the addition of potassium in the form of potassium chloride did not stimulate mannite-decomposition. Furthermore, since $K_2HPO_4$ in no instance caused mannite to break down more rapidly than $CaHPO_4$ alone or in combination with $CaCO_3$, we may conclude that the potassium ion seems to have no effect or only a very slight effect on that process. In 1914 in investigations of the conditions necessary for cellulose and peptone decomposition in the soil, the author (1) obtained similar results.

While the investigations referred to seem to prove that the speed of mannite-decomposition depends in the main on the reaction of the soil and its buffer content, as well as on its content of easily soluble phosphoric-acid-combinations, the present material also indicates that there are other important factors. We find, for instance, that the addition of $CaCO_3 + CaHPO_4$ does not stimulate mannite-decomposition equally in all soils. In the soil from Strøby, for instance, mannite-decomposition was complete at the end of 5 days while in soil from Lungaard a considerable portion of undecomposed organic matter remained in the tumbler after 10 days. In the soil from Slangerup (1917), which was drawn from unfertilized plots in an experiment with fertilizers, $CaCO_3$ alone or in combination with $CaHPO_4$ had no stimulating effect on decomposition. When $K_2HPO_4$ was added there was slight stimulation. That the content of potassium was not the important factor here may be seen from the fact that KCl had no effect when added to $CaCO_3 + CaHPO_4$. In a decomposition experiment made two years later with a new soil sample from the same plots, table 4, when carbonate of lime and calcium phosphate were added to the soil mannite decomposition was complete after 5 days.

There is reason, then, to expect that a determination of the power of soil to break down mannite may to some extent express its content of easily soluble phosphoric-acid-combinations and perhaps, too, of those readily assimilable by plants. As preliminary study of the question which is great importance

for practical soil investigations, an examination was made of certain soils whose need of phosphoric acid had been determined by field experiments carried out through many years. Unfortunately there are only a few experiments here in Denmark which can supply material for these investigations. This is much to be regretted both in the present work and in further studies which will aim at explaining the part played by the various substances in the soil.

The material used is from the following experiments.

Soil samples 1 and 2 from the permanent experiments with stable manure and artificial fertilizers on the "loamy field" at Askov Experiment Station (Field B 3) were a light loam rather dark in color. No. 1 was drawn from the plots which have been unfertilized since 1893. No. 2 was from plots fertilized only with superphosphates. From 1893 to 1907 85 kgm. of 18-per cent superphosphate was applied per Td[7] land per year. The following years 95 kgm. was applied annually. The sample was drawn in the autumn of 1919. A comparison between the plant production, (even though this was but scanty in both instances) on the phosphate fertilized and the unfertilized plots showed plainly that the soil in the latter plots was greatly in need of phosphoric acid.

Soil samples 3 and 4 from corresponding experiments begun at the same time on the "sandy field" at Askov Experiment Station (Field G-2) were light sandy soil long cultivated. Sample 3 was drawn from the unfertilized and sample 4 from plots fertilized only with superphosphates. The samples were drawn in the autumn 1918. Neither in this experiment nor in other experiments from the "sandy field" of Askov Experiment Station did the addition of superphosphate produce any effect (3).

Soil samples 5, 6, 7, and 8 from the "sandy field" of Askov Experiment Station were drawn during the winter of 1920 from an experiment with artificial fertilizers on alfalfa begun in its final form in 1899. As in the preceding experiment, this was a light sandy soil. Sample 5 was from an unfertilized plot, sample 6 from a plot treated with 150 kgm. Thomas phosphates per Td. land annually, sample 7 from a plot treated with 200 kgm. kainit per Td. land annually, and sample 8 from a plot treated with 200 kgm. kainit and 150 kgm. Thomas phosphates per Td. land annually. All the samples showed acid reaction, so the decomposition experiments were made both with and without the admixture of carbonate of lime (1 per cent of the weight of the soil) While the plant growth showed a considerable reaction from the addition of potassium, neither sample 6 nor sample 8 showed any reaction from the addition of Thomas phosphates (3).

Soil samples 9–10 were drawn from a starvation experiment on the demonstration field of the Royal Agricultural College in Copenhagen (9). The soil is a heavy loam, rich in humus. Sample 9 was drawn from plots which since 1898 have been fertilized annually with 30 kgm. nitrogen, (in the form of Chili saltpeter) and 30 kgm. potassium (in the form of potassium sulfate) per Td. land, and thus starved for phosphoric acid. Sample 10 was drawn from plots which in addition to the amount of nitrogen and potassium mentioned above, had been treated annually with 18-per cent superphosphates, containing 20 kgm. of $P_2O_5$. The samples were drawn in March, 1919. No effect from the addition of phosphates has been observed hitherto.

Samples 11 and 12 were drawn October, 1917, from a starvation experiment at Tystofte Experiment Station. The soil was a heavy loam, poor in humus. Sample 11 was drawn from plots which, since 1913, have been treated annually with an average of 175 kgm. Chili saltpeter and 138 kgm. 37-per cent potassium fertilizer per hectare and thus starved for phosphoric acid. Sample 12 was drawn from plots which, in addition to the fertilizers named above, had been treated annually with 200 kgm. 18-per cent superphosphates per hectare. There was a distinct effect of phosphoric acid starvation in the plots to which no phosphoric acid fertilizer was added, observable in the plant growth.

[7] A Td. (Tønde) land = 0.55 hectare.

Soil samples 13, 14, 15 and 16 were drawn in November 1920 from an experiment begun in the spring of 1916 at Smakkebakgaard near Lundby, Seeland. The soil is a rather heavy loam, which is very productive. The samples were taken from plots receiving the following treatments: 13, Chili saltpeter; 14, Chili saltpeter and superphosphates; 15, Chili saltpeter and potassium fertilizer; 16, Chili saltpeter, potassium fertilizer and superphosphate. All the crops, though particularly barley, on the plots represented by samples 14 and 16 showed considerable response to the phosphoric acid fertilizer.

Soil samples 17 and 18 were drawn in April, 1921, from an experiment begun in 1916 at Kalby, Seeland, on a very productive loamy soil. Plot 17 had been unfertilized while plot 18 had received treatment of superphosphate. No effect from the addition of superphosphates had been observed.

Soil sample 19: In an experiment field at Oelstykke, Seeland, on sandy soil, 300 kgm. of superphosphate per hectare as a supplement to 200 kgm. Norwegian saltpeter and 150 kgm. of potassium fertilizer increased the yield of swedes[8] by 15 per cent. In 1920, sample 19 from soil adjacent to the experiment was drawn to be used at the Royal Agricultural College in a pot experiment with barley. It was found that easily soluble phosphates (54 kgm. $P_2O_5$ per hectare) increased the crop by 11–19 per cent. At the close of the experiment, samples were taken from the pots which had received nitrogen-potassium fertilizer. Since the soil showed an acid reaction to litmus, lime was added to half of the samples (see further table 4).

Sample 20 was drawn in 1921 from the unfertilized plots of an experiment at Blaahøj, Grindsted (Jutland) on a dark gray, sandy soil rather rich in humus, but unproductive The experiment was begun in the spring of 1920, with swedes as the experimental crop. Plot treatments were: (a) unfertilized, (b) 200 kgm. Norwegian saltpeter, (c) 200 kgm. Norwegian saltpeter and 300 kgm. of 18-per cent superphosphates. We have results from the one year only, but the effect from superphosphates was so enormous that no further proof of the great phosphoric acid requirement of the soil was necessary. The addition of superphosphate more than quadrupled the yield. On account of the acid reaction, this sample was set aside both with and without lime. (Cf. table 4.)

The results of the investigations of these soils are given in table 5.

After the differences in the microörganism flora which cause mannite decomposition are removed by inoculation, the inoculated cultures express the influence which the differences in the *chemical* condition of the soil exert on mannite decomposition. *In those experiments in which the addition of phosphoric acid has considerably increased the yield, all the soil samples from plots not fertilized with phosphoric acid possess only a very slight power of mannite decomposition.* Out of this group, however, samples from plots in the three permanent experiments (Askov "loamy field," Tystofte and Lundby), which for several years had been treated with superphosphates, possess a very great power of mannite-decomposition. In the soil from Askov mannite-decomposition was well advanced after 5 days, and in all three soils it was complete after 10 days. In the two decidedly lime-requiring soils within this group (from Oelstykke and Grindsted) mnnite decomposed very slowly and the limed samples were only slightly ahead of the unlimed samples.

These results agree closely with the results from three of the four fertilization experiments *in which no effect was observed from the addition of superphosphates.* These three are the experiment with stable manure and artificial fertilizers on

[8] A swede is *Brassica Napus L. rapifera.*

TABLE 5

*Mannite decomposition in soils from field experiments with phosphoric acid fertilizer*

| SAMPLE NUMBER | ACID EFFERVESCENCE | LITMUS REACTION | DEVELOPMENT OF AZOTOBACTER | PHOSPHORIC ACID CONTENT | | EFFECT OF PHOSPHATE FERTILIZER IN FIELD | CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS† | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Uninoculated culture | | | | | | Inoculated culture | | | | | |
| | | | | $P_2O_5$ soluble in muriatic acid | $P_2O_5$ per liter of $CO_2$ saturated water | | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days |
| | | | | *per cent*‡ | *mgm.* | | *cc.*† | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* | *cc.* |
| 1 | v. weak¶ | alk.¶ | 4 | 0.046 | 0.26 | str.¶ | 39.8 | 37.6 | 36.7 | 37.5 | 35.7 | 35.4 | 40.4 | 38.1 | 36.8 | 35.9 | 30.3 | 25.3 |
| 2 | weak | alk. | 4 | 0.058 | 2.08 | | 38.7 | 7.7 | 3.1 | | | | 19.4 | 2.4 | | | | |
| 3 | weak | neut. sl. alk. | 4 | 0.078 | 1.56 | none | 37.8 | — | 4.7 | 3.0 | | | 2.9 | | | | | |
| 4 | weak | neut. sl. alk. | 4 | 0.092 | 2.42 | | 37.9 | 40.2 | — | — | — | 0.5 | 2.7 | | | | | |
| 5 | none | neut. sl. acid | 0 | 0.075 | 1.96 | none | | | | | | | 37.6 | 39.6 | 35.1 | 24.6 | 12.0 | |
| 6 | none | neut. | 0 | 0.088 | 4.20 | | | | | | | | 33.2 | 20.6 | 5.1 | | | |
| 7 | none | neut. sl. acid | 0 | 0.074 | 1.0 | none | | | | | | | 37.5 | 38.4 | 35.4 | 30.3 | 22.7 | |
| 8 | none | neut. sl. acid | 0 | 0.080 | 2.50 | | | | | | | | 37.2 | 38.1 | 33.7 | 29.0 | 20.0 | |
| 5a* | | | | | | | | | | | | | 33.8 | 14.8 | 2.8 | | | |
| 6a | | | | | | | | | | | | | 21.2 | 1.9 | | | | |
| 7a | | | | | | | | | | | | | 33.7 | 17.0 | 5.5 | | | |
| 8a | | | | | | | | | | | | | 33.1 | 16.2 | 2.3 | | | |
| 9 | weak | alk. | 4 | 0.137 | 1.10 | none | 32.5 | 5.6 | | | | | 3.6 | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | weak | alk. | 4 | 0.127 | 3.34 | | 19.0 | 5.7 | | | | | 3.8 | | | | | |
| 11 | none | neut. sl. acid | 0 | 0.050 | 0.48 | str. | 39.0 | 34.7 | 25.5 | 16.0 | 5.3 | | 38.6 | 33.6 | 22.0 | 14.3 | 3.6 | |
| 12 | none | neut. | 0 | 0.058 | 1.28 | | 38.9 | 34.7 | 22.0 | 7.7 | 3.9 | | 38.3 | 4.2 | | | | |
| 13 | none | neut. | 3 | 0.079 | 1.0 | r. str. | 38.6 | 36.8 | 35.0 | 23.3 | 8.5 | | 39.3 | 37.5 | 31.5 | 7.2 | 3.7 | |
| 14 | none | neut. | 4 | 0.089 | 2.08 | | 38.6 | 35.6 | 21.9 | 4.4 | 3.9 | | 37.3 | 2.9 | | | | |
| 15 | none | neut. | 3 | 0.080 | 0.94 | r. str. | 37.9 | 36.5 | 35.2 | 17.5 | 4.5 | | 37.9 | 35.8 | 28.8 | 6.4 | 3.6 | |
| 16 | none | neut. | 4 | 0.090 | 1.26 | | 39.1 | 28.8 | 4.2 | | | | 34.0 | 2.9 | | | | |
| 17 | str. | alk. | 4 | 0.071 | 3.40 | none | 20.3 | 6.4 | | | | | 13.5 | 3.9 | | | | |
| 18 | str. | alk. | 4 | 0.078 | 5.76 | | 35.6 | 4.1 | | | | | 10.6 | 2.5 | | | | |
| 19 | none | acid | 0 | 0.066 | 0.26 | r. str. | 38.8 | 36.5 | 35.0 | 30.2 | 13.4 | 4.9 | 38.7 | 36.1 | 34.8 | 30.9 | 19.2 | 8.7 |
| 20 | none | sl. acid | 0 | 0.013 | 0.87 | v. str. | 40.7 | 39.2 | 39.1 | 39.3 | 37.1 | | 40.0 | 39.9 | 39.1 | 39.5 | 38.1 | |

* 5a, 6a, 7a and 8a are same as 5, 6, 7 and 8 respectively with the exception that the soils were mixed with 1 per cent of calcium carbonate.

† See footnote to table 1. These data are also averages.

‡ 100 per cent = weight of air-dry soil.

¶ Abbreviations same as in previous table.

the Askov "sandy field," that on the field of the Royal Agricultural College and that at Kalby. Soil from plots to which no phosphoric acid was added possessed, in each case, great power of mannite-decomposition. In every case this was complete after 5–10 days. In these experiments there can be no question of a difference in speed of mannite-decomposition in soil samples fom the non-phosphoric acid fertilized and the phosphoric acid fertilized plots. In the experiment on Askov "Sandy field" mannite decomposition was very slow both in plots fertilized with phosphoric acid and in plots not fertilized with phosphoric acid. This is, undoubtedly, due to the decidedly acid reaction of the soil samples in question. When the soil samples were treated with carbonate of lime, mannite-decomposition was in every instance, comparatively rapid. Table 5 shows that soil from plots which received potassium-fertilizer only possessed, after the addition of lime, just as great a power of mannite-decomposition as soil in plots receiving both potassium-fertilizer and Thomas phosphates. On the other hand, strangely enough, soil fertilized with Thomas phosphates alone showed, in both series of experiments a greater power of mannite-decomposition than any of the other soil samples drawn. The cause of this may possibly lie in the fact that this soil contained a very large content of phosphoric acid in combinations soluble in carbonic acid. The saturation-concentration of phosphoric acid in the carbonic-acid extract varied greatly in this experiment; i. e., from 1.0 on the kainit plots, to 4.20 on the Thomas-phosphate plots. But even in soil from the kainit plots soluble phosphoric-acid combinations were found in quantities sufficient for both plant nourishment and rapid mannite-decomposition.

In the other experiments, too, there was a very considerable difference in phosphoric-acid content in carbonic-acid-extracts of soils from the non-phosphoric acid and phosphoric acid fertilized plots. This difference was relatively greatest in the experiment on Askov "loamy field." Furthermore the *saturation-concentration of $P_2O_5$ in carbonic-acid-extracts, both absolutely and relatively, was very small in all the five soils with distinct phosphoric-acid-requirement.*

The sensitiveness of the speed of mannite-decomposition to the lack of phosphoric acid in the soil appeared very plainly in the investigations of soil samples from experiments at *Tystofte* and *Lundby* (conducted for only a very few years). The amount of phosphoric acid which had been added to the superphosphate-fertilized plots during the years of the experiment and of which a large part may be considered to be removed with the crop, only included 180 and 245 kgm. per hectare. This is a very small percentage of the $2\frac{3}{4}$ million kgm. of soil in the ploughed surface, 20 cm. deep. The saturation-concentration of $P_2O_5$ in carbonic-acid-saturated water was very sensitive to these small additions of phosphates. In the experiments at Tystofte 0.48 mgm. of $P_2O_5$ per liter of carbonic-acid-saturated water was found in the soil not fertilized with superphosphates, and 1.28 mgm. in the soil fertilized with superphosphates. In the experiments at *Lundby* there was a difference of 1.08 mgm. of $P_2O_5$ between

Chili-saltpeter and Chili-saltpeter-superphosphates and 0.32 mgm. between Chili-saltpeter and to make up for potassium Chili-saltpeter-fertilizer-superphosphates. It is worthy of note that in the last instance in spite of the comparatively small difference in saturation-concentration of $P_2O_5$, the difference in speed of mannite-decomposition was great. This condition might indicate that mannite-decomposition expresses the assimilability of phosphoric acid in the soil both for the microörganisms in question and for the plant growth better than saturation-concentration expresses it. The condition found in sandy soil from *Blaahoj*, may be said to point in the same direction. This soil, which was probably the most phosphoric-acid-requiring soil of all these tested in these investigations, possessed a very slight power to break down mannite, while its saturation-concentration of $P_2O_5$ was comparatively high (0.87 mgm.).

This investigation, as well as the investigations on the variation in the power of mineral soils to break down mannite, seems to show that comparative investigations of the relation of mannite-decomposition to the soil's content of readily soluble phosphoric-acid-combinations can only be made on *non-lime-requiring soils*. In the case of lime-requiring soils it will be necessary to add an amount of carbonate of lime sufficient for a maximum decomposition-speed before the decomposition experiments are begun.

The results of investigations with "uninoculated" cultures point in the same direction as the results of investigations with the "inoculated" but do not express as sharply the difference in the soil's content of readily assimilable phosphoric-acid-combinations as these.

The general relation between the reaction of the soil and the solubility of the phosphoric-acid-combinations in the soil is shown in the first part of table 6 which does not include the soils (cf. p. 350–351 drawn from various experiments with phosphoric acid fertilizers and the soil samples from Seeland and the Faroe Islands, which differed greatly from the ordinary types of Danish soils. The second part of table 6 shows the relations between the reaction, the azotobacter development of the soil in the azotobacter test, and its content of lime and magnesia in combinations soluble in ammonium chloride. In this report only the Seeland and Faroe Islands soils are omitted. Of these the two from Seeland showed a very large magnesia-content as compared with the Danish soils (see table 2).

The *content of phosphoric acid in combinations soluble in muriatic acid*, did not differ markedly within the single reaction groups.

*There were much greater differences in the saturation-concentration of $P_2O_5$ in carbonic-acid-extracts.*

Among the loamy soils the saturation concentration of $P_2O_5$ is very small in soil with acid reaction and the variations are also comparatively small (0.17–1.14 mgm.); in the neutral lime requiring soils without azotobacter development, the content of phosphoric acid in the carbonic acid extracts is, on the average, much greater although still small and again with compara-

TABLE 6

*Relation of the solubility of phosphoric-acid-combinations in mineral soils, to their reaction and to azotobacter and content of lime-and-magnesia-combinations soluble in ammonium chloride*

| REACTION AND BASICITY | NUMBER OF SOILS | $P_2O_5$ IN COMBINATIONS SOLUBLE IN MURIATIC ACID | | | NUMBER OF SOILS | $P_2O_5$ PER LITER OF CARBONIC-ACID-SATURATED WATER (SATURATION CONCENTRATION) | | | NUMBER OF SOILS | CaO SOLUBLE IN AMMONIUM CHLORIDE | | | NUMBER OF SOILS | MgO SOLUBLE IN AMMONIUM CHLORIDE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Least | Greatest | Average | | Least | Greatest | Average | | Least | Greatest | Average | | Least | Greatest | Average |
| **Loamy soils** | | | | | | | | | | | | | | | | |
| | | *per cent** | *per cent* | *per cent* | | *per cent* | *per cent* | *per cent* | | *per cent* | *per cent* | *per cent* | | *per cent* | *per cent* | *per cent* |
| Acid | 9 | 0.063 | 0.137 | 0.090 | | 0.17 | 1.14 | 0.49 | 8 | 0.066 | 0.183 | 0.133 | 7 | 0.015 | 0.033 | 0.024 |
| Neutral without Azotobacter development | 7 | 0.058 | 0.134 | 0.098 | | 0.54 | 2.22 | 0.92 | 7 | 0.146 | 0.330 | 0.241 | 7 | 0.027 | 0.035 | 0.030 |
| Neutral with Azotobacter development | 12 | 0.051 | 0.144 | 0.091 | | 0.34 | 8.70 | 2.86 | 12 | 0.125 | 0.390 | 0.244 | 12 | 0.018 | 0.065 | 0.031 |
| Alkaline | 13 | 0.061 | 0.177 | 0.092 | | 0.46 | 10.0 | 2.71 | 12 | 0.282 | 1.110 | 0.534 | 12 | 0.015 | 0.032 | 0.023 |
| Without Azotobacter (lime-requiring soils) | 16 | 0.063 | 0.137 | 0.093 | | 0.17 | 2.22 | 0.68 | 15 | 0.066 | 0.330 | 0.183 | 14 | 0.015 | 0.035 | 0.027 |
| With Azotobacter (non-lime-requiring soils) | 25 | 0.056 | 0.177 | 0.091 | | 0.34 | 10.0 | 2.78 | 24 | 0.180 | 1.110 | 0.389 | 24 | 0.015 | 0.065 | 0.027 |
| **Sandy soils** | | | | | | | | | | | | | | | | |
| Acid | 9 | 0.021 | 0.117 | 0.070 | | 0.24 | 1.80 | 1.003 | 8 | 0.023 | 0.353 | 0.141 | 8 | 0.004 | 0.028 | 0.014 |
| Neutral without Azotobacter development | 8 | 0.064 | 0.123 | 0.086 | | 0.64 | 2.72 | 1.694 | 7 | 0.112 | 0.498 | 0.236 | 7 | 0.014 | 0.029 | 0.022 |
| Neutral with Azotobacter development | 9 | 0.048 | 0.098 | 0.072 | | 0.74 | 4.34 | 1.907 | 9 | 0.120 | 0.308 | 0.220 | 9 | 0.008 | 0.042 | 0.021 |
| Alkaline | 10 | 0.054 | 0.150 | 0.100 | | 1.55 | 12.24 | 4.013 | 10 | 0.332 | 0.924 | 0.575 | 10 | 0.012 | 0.029 | 0.021 |
| Without Azotobacter (lime-requiring soils) | 17 | 0.021 | 0.123 | 0.077 | | 0.24 | 2.72 | 1.328 | 15 | 0.023 | 0.498 | 0.185 | 15 | 0.004 | 0.029 | 0.018 |
| With Azotobacter (non-lime-requiring soils) | 19 | 0.048 | 0.150 | 0.087 | | 0.74 | 12.24 | 3.015 | 19 | 0.120 | 0.924 | 0.407 | 19 | 0.008 | 0.042 | 0.021 |

| | All soils | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acid | 18 | 0.021 | 0.137 | 0.080 | 0.17 | 1.80 | 0.75 | 16 | 0.023 | 0.353 | 0.137 | 15 | 0.004 | 0.033 | 0.019 |
| Neutral without Azotobacter development | 15 | 0.058 | 0.134 | 0.091 | 0.54 | 2.72 | 1.33 | 14 | 0.112 | 0.498 | 0.238 | 14 | 0.014 | 0.035 | 0.026 |
| Neutral with Azotobacter development | 21 | 0.048 | 0.144 | 0.083 | 0.34 | 8.70 | 2.38 | 21 | 0.120 | 0.390 | 0.234 | 21 | 0.008 | 0.065 | 0.022 |
| Alkaline | 23 | 0.054 | 0.177 | 0.096 | 0.46 | 12.24 | 3.28 | 22 | 0.282 | 1.110 | 0.553 | 22 | 0.012 | 0.032 | 0.022 |
| Without Azotobacter (lime-requiring soils) | 33 | 0.021 | 0.137 | 0.085 | 0.17 | 2.72 | 1.01 | 30 | 0.023 | 0.498 | 0.184 | 29 | 0.004 | 0.035 | 0.022 |
| With Azotobacter (non-lime-requiring soils) | 44 | 0.048 | 0.177 | 0.089 | 0.34 | 12.24 | 2.88 | 43 | 0.120 | 1.110 | 0.397 | 43 | 0.008 | 0.065 | 0.024 |

* 100 per cent = weight of air-dry soil.

tively small variations; in the neutral, non-lime-requiring soils with azotobacter development, the saturation concentration of $P_2O_5$ is on the average six times as great as in the acid soils and about three times as great as in the neutral soils without azotobacter development. The soils with strong alkaline reaction are very similar to the neutral non-lime-requiring soils with azotobacter development. If the material is classified as lime-requiring and non-lime-requiring soils as in table 6, it will be seen that the saturation concentration of $P_2O_5$ is about four times as great in the non-lime-requiring soils as in the lime-requiring soils. Another condition worthy of note is the fact that variations in the content of phosphoric acid in the extracts are much greater in the non-lime-requiring than in the lime-requiring soils. This indicates that *a certain lime-content is necesssary for the presence of any considerable amount of phosphoric-acid-combinations soluble in carbonic acid.* This fact may also be seen by comparing (table 6) the reaction of the soil and its content of lime soluble in ammonium chloride. Sandy soils revealed, on the whole, the same condition as loamy soils. It should be noted here that the saturation-concentration of $P_2O_5$ was, on the average, twice as large in both groups of lime-requiring soils as in the loamy soils. This may possibly express the fact that sandy soils are as a rule less prone to absorb phosphoric acid than loamy soils. The variation in the saturation-concentration of $P_2O_5$ in the carbonic-acid-extracts even in sandy soils was rather small in the two lime-requiring groups and much less than in the two non-lime-requiring groups in which the phosphoric-acid-content in the carbonic-acid-extracts was about $2\frac{1}{2}$ times as great as in the lime-requiring soils.

In consideration of the small number of soils and the very great variation within these groups in the content of phosphoric acid in the extracts, the very great difference present in the saturation-concentration in the two non-lime-requiring groups of soil in sandy soil may be assumed to rest to a certain extent on chance. Of all the soils examined, within the group of alkaline sandy soils, soil no. 89 has shown the largest amount of phosphoric-acid-combinations in carbonic-acid-extracts (12.2 mgm. $P_2O_5$ per liter).

The extremely small content of phosphoric-acid-combinations soluble in carbonic acid found in field soils with strong acid reaction, and the comparatively small variations in this content agree very well with the results of investigations made by C. W. Stoddart (7) in the United States and M. Weibull (8) in Sweden by which it was demonstrated that field soils with an acid reaction are almost always deficient in phosphoric acid. In general, the results indicate that the reaction of the soil and its buffer content determine very decidedly not only the immediate content of easily soluble phosphoric-acid-combinations in the soil but also its power to transform phosphates added into combinations difficultly soluble.

Three of the soil samples investigated (18, 29 and 75) were drawn from soils known to be very highly fertilized. They came from farms where for many years a large number of cattle have been fattened for market on a very rich

diet of oil cakes and cereals. Table 2 shows that these soil samples, both as regards the absolute content of phosphoric acid and the saturation-concentration of $P_2O_5$ in the carbonic-acid-extracts, were the richest in phosphoric acid within the reaction groups in question. Soil 75 was lime-requiring. Possibly that is why the saturation-concentration of $P_2O_5$ is much less than in the other two soils; but within the group of neutral soils without azotobacter development it shows the greatest content of phosphoric acid in carbonic-acid-extracts.

Investigations of the content of magnesia-combinations soluble in ammonium chloride showed with a very few exceptions (soil 36 from Vejlø, 38 and 39 from Seeland), that this is small and as a rule much smaller than the content of lime soluble in ammonium chloride. It was impossible to show a closer relation between the reaction of the soil and the content of magnesia similar to that which had been found for lime by the author both in this investigation and in earlier investigations (2).

## SUMMARY OF THE MAIN RESULTS

1. Observations seemed to show that important differences exist in the ability of various soils to decompose mannite.

2. A method for investigations of this kind was worked out.

3. An investigation was made of the variation in the mannite-decomposition power of soils expressed in the differences in speed of mannite decomposition.

4. *Both "uninoculated and inoculated" cultures were used,* in order to determine to what extent the variations found depended on differences in the chemical or microbiological nature of the soil. As a rule mannite-decomposition takes place more quickly in inoculated than in uninoculated cultures, but this difference is comparatively small, and *the differences in the power of soil to decompose mannite depend primarily on the differences in the chemical condition of the soil.*

5. A definite relation seemed to exist between the reaction of the soil (particularly its buffer action in the proximity of the neutral point measured by the author's azotobacter test) and its power of mannite decomposition.

6. No connection could be shown between the soil's content of phosphoric acid in muriatic-acid-soluble combinations, and its power to decompose mannite. On the other hand, experiments with *non-lime-requiring soils* proved by the azotobacter test to develop azotobacter showed that those among them having the greatest saturation-concentration of $P_2O_5$ in carbonic-acid-extracts possessed, as a rule, the greatest power to decompose mannite.

7. A study of chemical factors showed that the content of basic, buffer-acting lime-compounds and of easily soluble phosphoric acid compounds determine the speed of mannite-decomposition.

The results seem to indicate that a determination of the speed of mannite-decomposition in non-lime-requiring soils, gives information as to the soil's content of easily soluble phosphoric-acid compounds readily assimilated by plants in the same way as the author's azotobacter test expresses the need of the soil for lime. Further investigations are desirable.

8. A survey was made of the relations between the reaction of the soil and its content of phosphoric acid in muriatic-acid-soluble-combinations, the saturation-concentration of phosphoric acid in carbonic-acid-extract, and of the content of lime and magnesia in ammonium-chloride-soluble combinations.

N.B. After this paper was finished a special investigation showed that the method used in determining the content of organic matter could be made more complete. The method is therefore being subjected to careful experimental tests and the results will be published in a short time.

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# DETERMINATION OF ORGANIC MATTER IN DECOMPOSITION EXPERIMENTS WITH SOIL

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In making decomposition experiments in soil, the determination of soluble organic matter in soil extracts was applied for the first time, so far as is known, by one of the authors (1) of the present paper in his test for mannite-decomposition in soil. In the investigation, soil was mixed with 2 per cent of mannite and the progress of decomposition tested at stated intervals by determining the amount of soluble organic substances present due to the addition of mannite in an aqueous filtrated extract.

The method employed is described in full in the paper referred to and is based on the same principle as that commonly used in determining the amount of organic matter in drinking water. A portion of the filtrate to which sulfuric acid has been added is heated with a surplus of very dilute, about 0.02 N potassium permanganate, after which the amount of potassium permanganate necessary to oxidize the organic substances present is determined in the usual way with the help of oxalic acid.

It soon became apparent that the usual 10-minute decomposition-period used in testing water was insufficient to oxidize completely the maximum amount of organic matter (about 8 mgm. mannite) in the given portions of the soil extracts. Portions of 1–10 mgm. mannite dissolved in distilled water were measured off. To each was added 3 cc. dilute sulfuric acid, 50 cc. .02 *N* potassium permanganate solution and enough distilled water to make 63 cc. In each case the $KMnO_4$ concentration was the same. A special test showed the necessity of extending the decomposition period to 20 minutes and allowing oxidation to take place at not less than 80°C. This temperature was attained by plunging the beaker in which the reaction was taking place into boiling water. It was then possible to obtain both constant results and an entirely satisfactory agreement between the results of the replicate determinations.

The author later attempted a direct determination of mannite in the soil extracts. The results with the above method had not been wholly satisfactory and did not agree quantitatively with the equation:

$$C_6H_{14}O_6 + 13O = 6CO_2 + 7H_2O.$$

According to this equation, 71.4 cc. of .02 *N* potassium permanganate solution is required to oxidize 10 mgm. mannite, whereas in the experiment made only 36.4 cc. was used. A corresponding oxidation of but 1 mgm. mannite required 7.3 cc. permanganate or 102 per cent of the amount computed. Table 1 indicates that with increasing amounts of mannite the actual amount of permanganate used becomes steadily less than the computed amount.

This investigation seems to indicate very clearly that *a considerable surplus of potassium*

*permanganate is necessary to oxidize mannite completely to carbonic acid and water.* Decomposition occurred regularly as long as no more than 25–30 cc. permanganate solution was used with 4–5 mgm. mannite. The difference between the actual and the computed values at this point was still comparatively slight; i.e., 17–19 per cent. When greater quantities of mannite were used the difference was very considerable; with 6 mgm. about 30 per cent; with 8 mgm., about 41 per cent; and with 10 mgm., about 50 per cent.[1] In another experiment, 50 and 100 cc. permanganate solution were used to oxidize 10 mgm. mannite. Here the amounts of permanganate were used respectively 53 and 78 per cent of those computed.

These investigations suggested that more complete data on the relation between the amounts of mannite and potassium permanganate might make it possible to work out a quantitative method.

TABLE 1

*Relation between the amounts of mannite and potassium permanganate used*

| MANNITE | AMOUNT OF 0.02 *N* $KMnO_4$ SOLUTION USED | | | | |
|---|---|---|---|---|---|
| | Determinations | | | Average corrected for check | Relation to the amount computed |
| | a | b | Average | | |
| *mgm.* | *cc.* | *cc.* | *cc.* | *cc.* | *per cent* |
| 0 | 1.3 | 1.4 | 1.4 | | |
| 1 | 8.4 | 8.9 | 8.7 | 7.3 | 102.2 |
| 2 | 15.1 | 15.2 | 15.2 | 13.8 | 96.7 |
| 3 | 19.9 | 20.5 | 20.2 | 18.8 | 87.8 |
| 4 | 24.8 | 25.6 | 25.2 | 23.8 | 83.4 |
| 5 | 30.6 | 30.0 | 30.3 | 28.9 | 81.0 |
| 6 | 31.9 | 32.0 | 32.0 | 30.6 | 71.5 |
| 7 | 32.7 | 33.3 | 33.0 | 31.6 | 63.3 |
| 8 | 34.8 | 34.9 | 34.9 | 33.5 | 58.7 |
| 9 | 35.9 | 36.7 | 36.3 | 34.9 | 54.3 |
| 10 | 37.4 | 38.0 | 37.7 | 36.3 | 50.9 |

A few preliminary investigations on the effect of lengthening the heating period and the addition of various substances which might be supposed to have a catalysing effect and so further oxidation, showed that the object was not to be attained in this way. The effect of a 0.1 *N* and a 0.05 *N* solution instead of the 0.02 *N* potassium permanganate was tested. The amount of the 0.02 *N* solution used was not increased because it seemed desirable to avoid using unreasonably large amounts of titrating fluid.

It was then found that 0.1 *N* $KMnO_4$ gave unreliable results. Only half as much potassium permanganate solution was used in the separate portions after which the addition of distilled water brought the total amount of liquid in each up to 63 cc.

[1] Earlier investigations by the author on the use of $KMnO_4$ in the oxidation of pure mannite (1) showed that 10 mgm. mannite required 43 cc. 0.02 *N* potassium permanganate solution. In this investigation, however, liquid portions containing 6 mgm. mannite were used, after which the results for 10 mgm. were computed. This explains the lack of agreement between the two investigations.

Table 2 shows that small amounts of mannite (1 and 2 mgm.) required more of the 0.1 *N* permanganate solution than the *theoretical* amount. The cause of this has not yet been determined but titrating back again with a strong permanganate solution showed a clear change to be difficult due to the precipitation of $MnO_2$. With 0.02 *N* solution instead of 0.1 *N* on titrating back again, the agreement between the theoretical and actual values was much better. Expressed in percentage, the relation diminished gradually from 109.5 (1 mgm. of mannite).

A series of test with 0.05 *N* $KMnO_4$ in 50-cc. potassium permanganate portions was made, using 0.02 *N* solution for titrating back.

Table 3 shows some of the results. Here again the figure for the smallest amount of mannite used is too high, but the difference between the actual and

TABLE 2

*Relation between the amounts of mannite and potassium permanganate used*

| MANNITE | AMOUNT OF 0.1 *N* $KMnO_4$ USED (SAME SOLUTION USED FOR TITRATING BACK) | | | | |
|---|---|---|---|---|---|
| | Determinations | | | Average corrected for check | Relation to the amount computed |
| | a | b | Average | | |
| *mgm.* | *cc.* | *cc.* | *cc.* | *cc.* | *per cent* |
| 0 | 0.2 | 0.1 | 0.2 | | |
| 1 | 2.7 | 2.8 | 2.8 | 2.6 | 179.6 |
| 2 | 3.8 | 4.1 | 4.0 | 3.8 | 133.1 |
| 3 | 4.9 | 4.7 | 4.8 | 4.6 | 107.4 |
| 4 | 6.2 | 6.2 | 6.2 | 6.0 | 105.1 |
| 5 | 7.5 | 7.6 | 7.6 | 7.4 | 103.7 |
| 6 | 8.7 | 8.9 | 8.8 | 8.6 | 100.4 |
| 7 | 10.4 | 10.3 | 10.4 | 10.2 | 102.1 |
| 8 | 11.2 | 11.0 | 11.1 | 10.9 | 95.3 |
| 9 | 12.3 | 11.8 | 12.1 | 11.9 | 92.6 |
| 10 | 13.3 | 13.0 | 13.2 | 13.0 | 91.1 |

computed values is only comparatively small, and so far as the method is concerned it is of no importance. In any case the use of only about 7 cc. potassium permanganate will indicate that the decomposition is approximately complete, and at that point it is immaterial if the amount used is 1 cc larger. A content of 2–4 mgm. mannite showed practical agreement between the values found and computed, but when 8 mgm. mannite was present, which was about the maximum amount possible in the soil extracts examined,[2] the figure found is 7 per cent too low. It is therefore, better not to use quantities of the extract larger than those corresponding to a content of 4–5 mgm. mannite.

[2] The portions of soil extract measured off (10 cc.), correspond to 0.5 gm. moist soil (about 0.4 gm. air-dry). At the beginning of the experiment the soil contained 10 mgm. mannite per 0.5 gm. air-dry soil. The values actually found, then, are about 20 per cent lower than those computed. This relationship is of some importance in interpreting the results of earlier experiments.

By using 0.05 *N* potassium permanganate solution for the oxidizing agent, it is possible to determine the amount of mannite present in soils with a degree of accuracy sufficient for tests on the ability of soils to decompose mannite.

The modified method of procedure is as follows:

Of the soil extracts prepared,[3] place an amount corresponding to about 0.25 gm. soil in a 400-cc. beaker with 50 cc. .05 *N* potassium permanganate solution and 3 cc. diluted sulfuric acid (6:100). Heat this for 20 minutes by plunging the beaker into boiling water. Add 50 cc. 0.05 *N* oxalic acid solution and titrate with 0.02 *N* potassium permanganate solution.

The very detailed calculation necessary if the permanganate solution and the oxalic acid are not exactly 0.02 *N* may be avoided by using a check in which all errors in method are included. Then only one correction for the 0.02 *N* potassium permanganate solution is necessary.

TABLE 3

*Relation between the amounts of mannite and potassium permanganate used*

| MANNITE | AMOUNT OF 0.05 *N* $KMnO_4$ USED (0.02 *N* SOLUTION USED FOR TITRATING BACK) | | | | |
|---|---|---|---|---|---|
| | Determinations | | | Average corrected for check | Relation to the amount computed |
| | a | b | Average | | |
| *mgm.* | *cc.* | *cc.* | *cc.* | *cc.* | *per cent* |
| 0 | 1.6 | 1.6 | 1.6 | | |
| 1 | 9.8 | 8.7 | 9.3 | 7.7 | 107.8 |
| 2 | 16.4 | 15.5 | 16.0 | 14.4 | 100.9 |
| 3 | 23.2 | 22.6 | 22.9 | 21.3 | 99.5 |
| 4 | 29.5 | 30.7 | 30.1 | 28.5 | 99.8 |
| 5 | 35.8 | 35.9 | 35.9 | 34.3 | 96.1 |
| 6 | 41.8 | 41.7 | 41.8 | 40.2 | 93.9 |
| 7 | 48.5 | 47.8 | 48.2 | 46.6 | 93.3 |
| 8 | 55.4 | 53.7 | 54.6 | 53.0 | 92.8 |
| 9 | 62.2 | 61.7 | 62.0 | 60.4 | 94.0 |
| 10 | 65.2 | 65.9 | 65.6 | 64.0 | 89.7 |

Table 1 shows that the values obtained (taken both absolutely and relatively) from the method used in earlier investigations on the ability of soil to decompose mannite, were too low in the first stages of mannite decomposition. This does not, however, prevent a clear interpretation of results. A consumption of 35–40 cc. of 0.02 *N* potassium permanganate solution under the conditions of those investigations, must be considered an expression of the fact that only a slight or no decomposition of the mannite contained in the soil took place. A consumption of more than 30 cc. is an expression of the fact that the larger part of the mannite is still present in the soil. At the close of 2–3 periods,

[3] On the preparation of the extract see the author's (1) earlier article. To reduce the possibility of errors in analysis it is recommended to use 200 cc. water in preparing the soil extracts instead of 100 cc. In that case, 10 cc. of the filtrate should be measured off for analysis. If the relationship between soil and water used hitherto is kept, only 5 cc. of the extract is used.

(10–15 days), a content of organic matter present corresponding to more than 20–25 cc. expresses, in every case, the fact that mannite decomposition occurred very slowly.

Although, it is probable that no other conclusions would have been reached by using a more perfect method in determining the content of organic matter in soil extracts, yet it is evident that the results would appear more certain if expressed in values more nearly correct even from an absolute point of view. This study has revealed a method by which this is possible.

In connection with the investigations so far discussed, a few other experiments were made. The object of these was to explain the peculiar fact that only when a *considerable surplus* of $KMnO_4$ is used does complete oxidation of mannite to carbon dioxide and water take place. When this surplus is not present a very considerable amount of acetic acid, a substance not affected by permanganate, is formed. In such experiments 100 mgm. mannite was oxidized with 500 cc. of 0.02 *N* $KMnO_4$. When the precipitated $MnO_2$ has been filtered out, the filtrate is neutralized with carbonate of soda, evaporated, led over into a fractionally graded retort, phosphoric acid added and then distilled. It was very easy to make a qualitative determination of the presence of acetic acid. A titration of a part of the distillate will show considerable acidity. That a very greatly reduced consumption of $KMnO_4$ appears when mannite is oxidized to acetic acid is readily explainable. If extreme oxidation is carried on according to the equation:

$$C_6H_{14}O_6 + O = 3C_2H_4O_2 + H_2O$$

only one-thirteenth of the amount of permanganate necessary when a complete oxidation to carbon dioxide and water takes place, will be used.

In this connection we may add that direct experiments have shown that *n*-butyric acid is not affected by potassium permanganate. This fact is important for we may suppose that in mannite decomposition acetic acid and *n*-butyric acid as well as carbonic acid are formed. Lactic acid and isobutyl-alcohol, two products also resulting from mannite decomposition are readily oxidized by $KMnO_4$. According to our present knowledge of the course of decomposition these two latter substances will appear in much smaller quantities, only slightly affecting the amount of permanganate used. This amount depends for the most part on the amount of *undecomposed mannite*. Since direct mannite determinations are difficult and since, to the author's knowledge, there is no exact method for a quantitative determination of such small quantities as these experiments deal with, this fact is of great importance in the interpretation of the experimental method worked out here.

## SUMMARY

Investigations of the possibility of a direct determination of mannite in soil extracts showed that the method hitherto used in determining organic sub-

stances in the soil extracts is not entirely satisfactory, for the oxidation of the greatest amount of mannite able to appear did not occur quantitatively according to the equation:

$$C_6H_{14}O_6 + 13\ O = 6CO_2 + 7H_2O.$$

This result was made the subject of a special investigation. It was found that a *very considerable surplus of potassium permanganate* is necessary for a quantitative oxidation.

A modified method for the determination of organic matter is recommended.

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# REVERSION OF ACID PHOSPHATE IN ACID SOILS

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In an attempt to gain some knowledge of the fate of superphosphate when applied to acid soils with and without the addition of limestone, a series of mixtures of some samples of acid soil, superphosphate and limestone were made, allowed to stand for a month, and then analyzed for "available" phosphoric acid.

## MATERIALS USED

Two of the most acid soils encountered by this station were used, viz., soil X and soil Y.

Soil X is a brown sandy loam, with a lime requirement of 3713 pounds of $CaCO_3$ per acre (Veitch).

Soil Y is a dark grey, very light, peaty soil containing a large amount of organic matter with a lime requirement of 3740 lbs. $CaCO_3$ per acre. This soil had an abnormally high water-holding capacity of about 200 per cent.

Soil X contained 0.21 per cent lime and soil Y 0.34 per cent.

The superphosphate used contained 14.37 per cent water-soluble and 14.94 per cent citric-acid-soluble phosphoric oxide.

Two grades of limestone were used, A which passed a 1 mm. mesh sieve and contained 94.4 per cent calcium carbonate, and B which passed a 3 mm. mesh sieve, but not a 1 mm. mesh sieve and contained 74.3 per cent calcium carbonate.

The acidity of the soils was calculated to be equivalent to 1.5 gm. $CaCO_3$ per kilogram of soil which is equivalent to 2.1 gm. of the "medium" or 1.6 gm. of the "fine" limestone. The acidity of the superphosphate was found to be equivalent to 576 gm. of the "medium" or 428 gm. of the "fine" limestone per kilogram.

## MIXTURES MADE

Mixtures were made of 1 kilogram of air-dry soil, 25 gm. of superphosphate and varying amounts of limestone. After being moistened, the mixtures were stored in the dark in rubber-ringed mason jars for a month.

The two series of mixtures were made equally damp by adding water to 70 per cent of their capacity.

After a month the equivalent of 100 gm. of air-dry soil was weighed out from each mixture and the "available" phosphoric oxide extracted by the 2-per-cent citric acid method and determined by the volumetric molybdate method.

*Mixtures made with Soil X and "fine" limestone A*

1. 1 kgm. soil with 25 gm. superphosphate.
2. Limestone to satisfy the requirement of 1 kgm. of soil and 25 gm. superphosphate was mixed with 25 gm. of superphosphate which was then added to 1 kgm. of soil.
3. 1 kgm. soil with limestone calculated to satisfy the requirement of the soil and 25 gm. of superphosphate and then 25 gm. superphosphate mixed in.
4. 1 kgm. soil with 25 gm. superphosphate and then the limestone to satisfy the requirement of both soil and superphosphate mixed in.
5. 25 gm. superphosphate mixed with limestone to satisfy its requirement and then mixed with 1 kgm. of soil.
6. 1 kgm. of soil mixed with the limestone requirement of 25 gm. superphosphate and then with 25 gm. of superphosphate.

*Mixtures made with Soil X and "medium" limestone B*

7. Same as 2.
8. Same as 4.

*Mixtures made with Soil Y and "fine" limestone A*

9. Same as 1.
10. Same as 2.
11. Same as 3.
12. Same as 4.

*Mixtures made with Soil Y and "medium" limestone B*

13. Same as 6.

TABLE 1

*Percentage reversion of acid phosphate in acid soils*

| MIXTURE NUMBER | PHOSPHORIC OXIDE | | REVERSION |
|---|---|---|---|
| | In original mixture | After 30 days | |
| | *per cent* | *per cent* | *per cent* |
| 1* | 0.364 | 0.275 | 24.46 |
| 2 | 0.360 | 0.270 | 25.00 |
| 3 | 0.360 | 0.270 | 25.00 |
| 4 | 0.360 | 0.270 | 25.00 |
| 5 | 0.360 | 0.260 | 27.89 |
| 6 | 0.360 | 0.255 | 29.29 |
| 7 | 0.358 | 0.245 | 31.68 |
| 8 | 0.358 | 0.250 | 30.29 |
| 9 | 0.364 | 0.145 | 60.16 |
| 10 | 0.360 | 0.140 | 61.11 |
| 11 | 0.360 | 0.140 | 61.11 |
| 12 | 0.360 | 0.145 | 59.72 |
| 13 | 0.359 | 0.135 | 62.43 |

* These two mixtures were unlimed.

From these figures it would appear that there is no actual benefit, as far as the availability of the superphosphate is concerned, to be had from mixing limestone with superphosphate or applying them to the soil separately even in different order.

With both soils the limed mixtures lost slightly more than the unlimed mixture except for mixture 12. Those mixtures limed with the coarser grade limestone seem, however, to have reverted most. It might be thought that the coarser grade limestone contained impurities that had made it more resistant to grinding and that these impurities caused the greater reversion of the phosphate. Analysis showed, however, that whereas the fine grade contained 4.53 per cent of iron oxide the medium grade contained only 0.26 per cent. A comparison of analyses made of two limestones—sieved into three grades after grinding—showed the amount of iron oxide to be greatest in the fine grade and least in the coarse. The lime content was just the opposite, the coarsest grades had the highest amount.

TABLE 2

*Partial composition of untreated soils*

| SOIL | $Fe_2O_3$ | $Al_2O_3$ | $P_2O_5$ | CaO | VEITCH LIME REQUIREMENT |
|---|---|---|---|---|---|
| X | 1.58 | 1.65 | 0.06 | 0.21 | 3713 |
| Y | 5.67 | 9.79 | 0.20 | 0.34 | 3740 |

How far the conditions of the experiment have affected the results is not known, but these figures certainly do not show any benefit a month afterwards from liming the soil when the amount of phosphate reverted is taken as the criterion. There is a large difference in the percentage of reversion in the two soils, although their lime requirements are the same when measured by the Veitch Method. The lime requirements of the two soils also agreed very closely when measured by the method of shaking the soil with alcoholic potassium thiocyanate and titrating the red color obtained with caustic soda. This method shows that the solubility of the iron in each soil is practically the same, and the different rates of reversion in the two cases are therefore, not due to a greater proportion of soluble iron in the one case.

A subsequent analysis showed that the soil having the most total iron caused the most reversion. Unfortunately it also had the most alumina as shown in table 2. Soil Y containing the greatest amount of iron oxide and alumina showed the greatest reversion of the phosphate despite the addition of lime. Comparing the amounts of these substances in the two soils, the reversion is not as great in Y as might be expected when the reversion in X and its composition are considered.

# AVAILABILITY OF ADSORBED PHOSPHORUS

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There is no question but that both the positive and negative ions of various salt solutions are adsorbed by soil colloids. Work by Gordon and Starkey (3) and by Lichtenwalner, Flenner and Gordon (1) has demonstrated this fact. Also, these studies showed that the salts adsorbed by the soil colloids of alumina and ferric oxide are only about one-third removed by the usual leaching process. This raised the question, whether the salts, which are adsorbed by these colloids and which cannot be dislodged by washing, are available to the plant when needed or are they forever locked in the soil in an unavailable form? The present investigation was taken up to throw some light on this question.

Colloidal ferric oxide and alumina were prepared as previously described. The colloids were allowed to suffer their maximum adsorption in a 0.05 *N* potassium acid phosphate solution. They were then subjected to washings until the filtrate gave no test for the adsorbed phosphate. On analysis, these gels were found to contain about one-third of their original phosphate.

Sweet potato plants were used because of the ease with which the roots might be divided between the two jars used in each experiment. Seed potatoes were sprouted in sand. Sprouts about six inches long were transferred to double jars consisting of two 250-cc. glass jars firmly bound side by side so they retained the same position relative to each other. The numbers 1–8 refer to double jars and 1a and 1b, 2a and 2b, etc. refer to the individual jars. Nutritive solutions were prepared and used as recommended by the National Research Council (2). Two sets of four double jars filled with white quartz sand were used. Colloidal ferric oxide which contained adsorbed unleachable phosphate was thoroughly mixed with the sand in 2b, 3b and 4b and colloidal alumina with adsorbed unleachable phosphate was mixed with jars 6b, 7b and 8b. The seedlings were weighed and one planted in each double jar. Half of the roots were placed in one side of the double jar and half in the other.

Jars 1a and 2a received distilled water; jars 1b 2b, 3a and 3b, a complete fertilizer solution minus phosphorus; jar 4a, a complete nutrient solution while 4b had a complete nutrient solution minus phosphorus. Jars 5–8 were treated in the same way.

After the plants had grown for eight weeks it was found that the roots filled the entire glass jars. The plants were then taken up, weighed and analyzed for phosphorus. The data are given in table 1.

Plant 1 ceased to grow when planted in jar 1 and showed a slight decrease in phosphorus content. Plant 5 also lost in phosphorus content, but in-

creased slightly in weight. Since neither of these double jars contained phosphorus, the migration of the phosphorus would naturally be outward. Also, the lack of phosphorus seemed to almost completely inhibit the growth of the plant. Plants 2 and 6 showed a marked increase in the weight of the plant and in phosphorus content. These plants had no source for phosphorus except adsorbed unleachable phosphorus. Plants 3 and 7 made a slight gain in phosphorus over 2 and 6 respectively, although they had access to

TABLE 1

*Growth of plants and phosphorus content*

| PLANT NUMBER | WEIGHT OF ORIGINAL PLANT | WEIGHT OF MATURE PLANT | GAIN IN WEIGHT | P IN ORIGINAL PLANT | P IN MATURE PLANT | GAIN IN P CONTENT |
|---|---|---|---|---|---|---|
| | *gm.* | *gm.* | *gm.* | *mgm.* | *mgm.* | *mgm.* |
| *Ferric oxide colloid* | | | | | | |
| 1 (control) | 1.70 | 1.70 | 0.00 | 1.00 | 0.9 | −0.1 |
| 2 | 2.55 | 19.10 | 16.55 | 1.49 | 6.74 | 5.25 |
| 3 | 3.40 | 30.20 | 26.80 | 1.99 | 10.22 | 8.23 |
| 4 | 2.20 | 34.40 | 32.20 | 1.29 | 20.76 | 19.47 |
| *Alumina colloid* | | | | | | |
| 5 (control) | 3.10 | 6.40 | 3.30 | 1.82 | 1.65 | −0.17 |
| 6 | 2.10 | 13.90 | 11.80 | 1.23 | 4.20 | 2.97 |
| 7 | 2.10 | 13.50 | 11.40 | 1.23 | 4.73 | 3.50 |
| 8 | 2.05 | 14.35 | 12.25 | 1.20 | 7.82 | 6.62 |

no more phosphorus. Plants 4 and 8 made a marked gain in both weight and phosphorus content. This would be expected since they had phosphorus in the nutrient solution in addition to the adsorbed phosphorus. At first thought this might seem to show that the plant could not get sufficient phosphorus for its natural growth from the unleachable adsorbed phosphorus yet the authors wish to do further work before making this statement.

This investigation has shown that phosphorus which has been adsorbed by soil colloids and which cannot be leached out by water is available for plant food.

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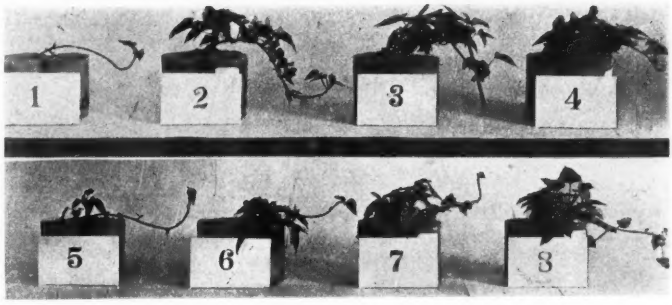


SWEET POTATO PLANTS AT COMPLETION OF EXPERIMENTS

# THE EFFECT OF DIFFERENT REACTIONS ON THE GROWTH AND CALCIUM CONTENT OF OATS AND WHEAT[1]

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In a previous investigation (2) the author reported the effect of different reactions on alfalfa and clover. This work was later extended to include the effect of different reactions on the growth and calcium content of oats and wheat. These plants were included, because of their importance as crops, and because of the lack of definite information along this line concerning them. The results of this investigation are reported in this paper.

It is generally recognized among agronomists that the cereals are less sensitive to acidity than most of the cultivated legumes. This is possibly due to the greater amount of basic material required for the normal growth of the legumes than for the cereals as suggested by Truog (15). Hartwell and Pember (5), (6) working with some of the cereals reported that there is a wide difference in the behavior of these plants toward acid soils. They found the order of sensitiveness to soil acidity, starting with the most sensitive, to be barley, wheat, oats and rye. When grown in solution cultures of varying degrees of acidity, they noted no appreciable difference in the behavior of these plants. They concluded that the presence of toxic aluminum salts and not acidity directly, caused the difference in behavior of the plants. They also reported (5) that acidity was more toxic to the growth of these plants than an equal alkalinity. Hoagland (7), Conner (3) and others later reported the reverse of this for barley, and Salter and McIlvaine (11) reported the reverse for wheat. The work of Conner (3), and Hartwell and Pember (5), (6) was largely concerned with the part played by aluminum in acid soils, which is probably an important factor in some cases.

Tarr and Noble (13) reported that wheat produced a maximum growth in solution cultures at a reaction of about pH 4. This does not seem to be exactly in accord with the results of other investigators (3), (5), (11).

The recent investigations by True (14) concerning the function of calcium in the nutrition of seedlings, and Truog (15), (16) concerning the feeding power of plants have shown the necessity of calcium as a nutritive element, as well as an amendment to acid media to induce a desirable assimilation of the other nutritive elements. A deficiency of calcium as a plant food in many acid soils has been reported by Shedd (12) MacIntire, (10), and others.

In general the data indicate that the cereals are less sensitive to acids than to alkalies, and also less sensitive to acids than most of the legumes. It

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station. The greenhouse work in connection with this investigation was done at the University of Wisconsin and the chemical analysis at the University of West Virginia.

The author wishes to express his appreciation for the helpful suggestions and criticisms tendered by Professors E. Truog and E. B. Fred.

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appears that wheat is more sensitive to acid media than oats, but no exact effect of reaction on these plants is given. The reaction of the medium in which the plants are grown seems to have a direct influence on the assimilation of the nutritive elements.

## EXPERIMENTAL

The Kherson oat and Marquis wheat were used in this investigation. All the experiments were carried out in quartz cultures, using the same containers and methods of growing the plants as described in the previous publication (2). The nutrient solution consisted of Crone's normal solution plus $\frac{1}{4}$ gm. of di-sodium phosphate per liter. The sodium phosphate was added to increase the buffer action of the solution. Varying amounts of dilute sulfuric acid and sodium carbonate were added to portions of this solution to obtain the different reactions of approximately pH 3, 4, 5, 6, 7, 8, 9 and 10. The

TABLE 1

*The growth and calcium oxide content of oats and wheat in sand cultures with Crone's nutrient solution at different reactions*

| APPROXIMATE REACTION | OATS | | WHEAT | |
|---|---|---|---|---|
| | Dry weight of 4 plants | Content of CaO | Dry weight of 4 plants | Content of CaO |
| *pH* | *gm.* | *per cent* | *gm.* | *per cent* |
| 3.3 | 2.4 | | 2.8 | |
| 3.9 | 4.4 | | 2.8 | |
| 5.0 | 8.8 | 0.57 | 6.5 | 0.51 |
| 6.0 | 10.7 | | 9.5 | |
| 6.9 | 10.0 | 0.52 | 9.4 | 0.52 |
| 7.8 | 5.3 | | 5.1 | |
| 8.8 | 4.3 | 0.40 | 3.8 | 0.51 |
| 9.6 | 1.4 | | 0.9 | |

acid was used to adjust the reactions in the acid range and the carbonate in the alkaline range. The solutions were renewed daily as previously described (1, 2) in order to keep the reactions as constant as possible.

The seedlings were allowed to grow at the different reactions for 2 months. The sand was then washed from the percolators and the photographs made of the entire plants. The plants were dried and the calcium determination made as previously described for alfafa and clover (2). All the experiments were carried out in duplicate, and the results are reported as averages. Table 1 and plate 1 give the results and growth of the plants at the different reactions.

Reference to plants grown at the different reactions will be made by referring to pH 3, 4, 5, 6, 7, 8, 9 and 10 although the actual pH values may have been slightly more or less. The average pH values are given in table 1.

It will be noted from this table that the maximum growth of oats took place at about pH 6, while that of wheat took place at a slightly less acidity. The

oat plants produced a much better growth at pH 4 and 5 than did the wheat. There was a considerable retardation of both the oats and wheat at pH 3, 4, 9 and 10. The retardation of wheat was greater than that of oats. No stooling or branching of any of the plants took place at pH 3 and 10. One plant each of the oats and wheat was almost dead at pH 10. A decrease in acidity from pH 5 produced a decrease in the calcium content of the oats, but no appreciable difference in the calcium content of the wheat at the different reactions was found. Four determinations of calcium were made at the pH values indicated and the results represent the averages.

## DISCUSSION

The better growth of the oats in the acid reactions than that of the wheat is possibly due to the greater normal acidity of the sap of the oat plants than that of the wheat. The normal acidity of the oat sap is about pH 5.6, while that of wheat is about pH 6.2 (4). The distinctly acid reaction of the oat sap, and the small amount of calcium required for its normal growth are no doubt important factors influencing the calcium in the plant. It is possible that the greater sodium content of the more alkaline solutions partially replaced the calcium in the oat plants with increase in alkalinity. Meyer (9) noted a decrease of calcium in the oat plant when grown on soils of high lime content and presumably less acid.

Since the sap of the wheat plant has about the same reaction as that of alfalfa and clover, and requires less than one-half the amount of calcium for its normal growth, the influences of reaction on the power of this plant to assimilate calcium would seem to be comparatively small, as was actually found in this investigation. On the other hand, a reaction more acid than the sap of the alfalfa and clover plants decidedly decreased the power of these plants to secure their necessary amount of calcium, as was shown in the previous investigation (2). The maximum growth of oats and wheat took place at a slightly acid reaction of pH 6 to 7, while that of alfalfa and clover took place at a neutral or slightly alkaline reaction of pH 7 to 8.

The results with wheat in this investigation do not agree exactly with those of Tarr and Noble (13) who reported that wheat produced a maximum growth in solution cultures at about pH 4. Salter and McIlvaine (11) reported a maximum growth of wheat at about pH 6, and of corn at about pH 5. Field observations indicate that wheat is less acid tolerant than corn. The different results obtained by Tarr and Noble (13) may be due to the composition of the nutrient solution used. It is very improbable that wheat will produce a maximum growth in soils at a reaction of pH 4.

There are undoubtedly other factors which affect the power of plants to assimilate the necessary nutritive elements, but it seems that the reaction of the medium exercises a very important influence. This possibly applies to all the plant nutrients as well as to calcium. The acidities which were injurious

to the oat and wheat plants in this investigation were less than that of many acid soils. The effect of acid soils on plant growth will be given in a later report.

## SUMMARY AND CONCLUSIONS

Oat and wheat plants were grown for two months in quartz cultures at different reactions. The nutrient solutions were changed daily in order to keep the reactions as constant as possible. The results may be summarized as follows:

1. The maximum growth of the oat plants took place at about pH 6, and that of wheat at a slightly less acidity of pH 6 to 7.
2. The oats grew at pH 4 and 5 much better than did the wheat. In general the oats were less affected by acidity than the wheat.
3. The oats and wheat produced practically no growth at pH 3 and 10, which are near their critical reactions.
4. An increase in acidity or alkalinity from the range pH 6 to 7 produced a decrease in the growth of both the oat and wheat plants.
5. A decrease in acidity from pH 5 produced a decrease in the calcium content of the oat plants, but not of the wheat plants.
6. The acidities which were injurious to the growth of the oats and wheat in this investigation are no greater than that of many acid soils.

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## PLATE 1

FIG. 1. OATS GROWN AT DIFFERENT REACTIONS IN QUARTZ SAND WHICH WAS REMOVED FOR MAKING THE PHOTOGRAPH
The numbers represent the approximate pH values

FIG. 2. WHEAT GROWN AT DIFFERENT REACTIONS IN QUARTZ SAND WHICH WAS REMOVED FOR MAKING THE PHOTOGRAPH
The numbers represent the approximate pH values


FIG. 1

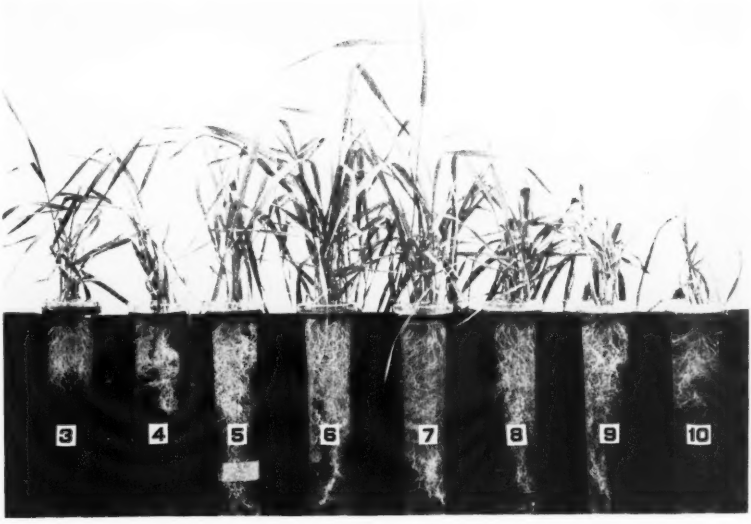


FIG. 2

# EFFECT OF ADSORPTION AND OTHER FACTORS ON CERTAIN PLANT FOOD CONSTITUTENTS OBTAINED IN THE DILUTE NITRIC ACID DIGESTION OF SOILS AND AN IMPROVEMENT FOR THEIR ESTIMATION[1]

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## INTRODUCTION

It is generally conceded that plant food exists in soils in two forms. One is often referred to as easily soluble, active or available, and consists of that smaller portion which plants can utilize for immediate needs as distinguished from the larger but less available reserve supply in the soil. The latter, of course, can be converted into the former by proper treatment of the soil. The distinction between the two forms and the use made of them by plants is well illustrated in the cultivation of virgin or old sod land in such crops as tobacco or potatoes for one or two seasons. Experience shows that this practice rapidly impoverishes the soil especially if the same crop is grown continuously without fertilization. This is probably due to the rapid removal of the more easily soluble plant food. The result is that the soil, although it may still contain an ample supply of total plant food and be free of diseases peculiar to these crops, nevertheless has to be restored somewhat to its virgin state before equivalent yields can be obtained. In other words, the more easily soluble plant food removed must be replaced. The problem, therefore, is to accomplish this as well as the natural agencies did before cultivation, and in less time, if possible.

## HISTORICAL

Numerous procedures have been used for the estimation of the easily soluble plant food in soils. The most popular is the weak acid digestion. The mineral acids, hydrochloric or nitric, are employed in this country whereas citric acid has found favor abroad.

Any laboratory method that might be proposed for this purpose is more or less arbitrary notwithstanding that results obtained by its use may be corroborated by field tests. For this reason, considerable discussion has arisen as to the value of any such method. Opponents contend that it is impossible to duplicate soil conditions in the laboratory, therefore no credence can be placed in such work. To support this they cite the fact that different methods often give widely different results on the same sample. Some even maintain that

[1] Published by permission of the Director of the Kentucky Agricultural Experiment Station.

[2] The writer desires to thank Dr. A. M. Peter, Head of the Department of Chemistry, for helpful criticism during this investigation.

all chemical methods, including those for the estimation of total plant food constituents, give no reliable data for indicating the productive capacity of soils. Other workers assert that some of the methods, do give results of a certain value when properly interpreted. They cite the fact that their findings have often been verified in the field and for this reason they continue to employ them in their work.

It is not necessary to discuss the relative merits of these opposite views or to include references to same in the literature as they are very voluminous. For this reason, only those which refer to certain matters discussed here are mentioned.

The most widely used methods in this country are those based on digesting the soil either in 0.2 *N* hydrochloric or nitric acid for a limited time at a definite temperature. The former was adopted by the Association of Official Agricultural Chemists (2) as a provisional method for easily soluble phosphate in soils. The literature at hand shows that the first work in this country with 0.2 *N* nitric acid as a solvent was done by Dr. A. M. Peter, at this Station (1, p. 77–80). This method was later recommended as a substitute for the 0.2 *N* hydrochloric acid method (3). It should be stated, however, that the method used and recommended at that time differs materially in some respects from the procedure as carried on in this work.

The last committee appointed by this Association for the revision of methods has failed, for some unaccountable reason, to include any method for the above separation, consequently none appears in the latest revision of "Methods of Analysis" (4).

Russell and Prescott (6) have made very extensive experiments on the determination of phosphorus obtained in the weak acid digestion of soils. Their work includes only this element and consists of a study of its adsorption by the soil and the effect of time of digestion and different strengths of various acids on the results obtained. They show that a short digestion with some acids, especially nitric acid not exceeding a certain strength, puts more phosphorus into solution than does one for a longer time. This they prove to be due to adsorption. On account of the different methods of procedure, different strengths of acid employed and the limited number of soils used in their work, their conclusions regarding the behavior of nitric acid have been only partly confirmed here. An excellent summary of some previous investigations on soil adsorption is given by these writers and in a résumé by Prescott (5).

During the course of some experiments on the digestion of soils with 0.2 *N* nitric acid early in 1916, the writer observed that adsorption apparently had considerable influence on the results obtained especially in the phosphorus determinations. These experiments were continued so as to include a large number of different types of soil and the estimation of elements other than phosphorus.

It was soon observed that short periods of digestion, for example, 5 minutes, showed as much or more phosphorus in solution in some soils as the regular 5 hour period. The short digestion also gave nearly as much potassium and calcium. All soils, however, did not behave the same in this respect. The results apparently showed that the effect of adsorption in some soils in the long digestion was a factor which could not be disregarded. As a result of this work, a short qualitative method for testing for easily soluble phosphate in soils was devised and published (7). In that paper, experiments are recorded which clearly show that the short 0.2 *N* nitric acid digestion is valuable for indicating the needs of a soil for phosphorus and in the present one others are given which extend its application to other plant-food constituents.

## METHODS

### *Fifth normal nitric acid method*

The procedure was to digest the air dried soil of 2 mm. or less in fineness in the proportion of 1 gm. of soil to 10 cc. of 0.2 *N* $HNO_3$ for 5 hours at room temperature, shaking every 30 minutes. If the sample contained carbonate

correspondingly stronger acid than the above was used so as to neutralize it without increasing the volume. After the digestion period the mixture was shaken again and poured on a large folded filter. The first 100 cc. of filtrate was poured back twice. The filtration usually required from 20–30 minutes when 120 gm. of soil was used. An aliquot was evaporated to dryness. More $HNO_3$ was added to oxidize organic matter, the solution was again evaporated and the last trace of $HNO_3$ eliminated by evaporation with HCl. The residue was dried on the steam bath to dehydrate $SiO_2$, taken up with HCl and $H_2O$, filtered and made to a definite volume. Separate aliquots corresponding to 40 gm. of soil were used for the determination of phosphorus and potassium and to 5 or 10 gm. for calcium. For those soils which contained large amounts of phosphorus, the aliquot represented 5 or 10 gm. of soil.

Phosphorus was determined with ammonium molybdate by precipitating at 40°C. in a small volume and allowing the yellow precipitate to stand at that temperature for an hour or so and finally over night at room temperature. It was then filtered, washed, dissolved in an excess of standard alkali and determined volumetrically by titration with standard acid.

Potassium was determined gravimetrically by evaporating an aliquot directly with $H_2PtCl_6$, filtering and washing the precipitate with acid alcohol (1 part concentrated HCl to 10 parts 95 per cent $C_2H_5OH$) and $NH_4Cl$ solution to remove impurities and finally with 80 per cent alcohol.

Calcium was determined by precipitating it as $CaC_2O_4$ with hot $(NH_4)_2C_2O_4$ solution added to the boiling solution previously slightly acidified with HCl. The whole was boiled for 2–3 minutes, and allowed to stand over night. The precipitate was then filtered, washed, heated with dilute $H_2SO_4$, and the calcium determined volumetrically with standard $KMnO_4$ solution.

Silicon was determined in the usual manner by treating the weighed residue of dehydrated silica obtained in the method with hydrofluoric acid and a little sulfuric acid to eliminate impurities.

### *Short fifth-normal nitric acid method*

Two procedures were used. In one, the soil was digested 5 minutes, shaking every minute, before pouring upon the filter. In the other, the soil was put upon the filter and the acid poured through it. The latter is designated in the tables as "Digestion = 0 minutes." In other respects, both were similar to the 5-hour extraction. Except where noted, 120 gm. of air-dry surface soil was used and where comparisons have been made the digestions were carried on together on equal quantities of soil. Some of the results in the tables are averages. Most, however, are individual determinations. As no particular difficulty was experienced in obtaining fairly close duplicates, it was not thought necessary to make them in every case.

### *Effect of time on the acid digestion*

Some experiments have been made in which the soil was digested in 0.2 *N* $HNO_3$ for different periods. The results are given in table 1.

### COMPARISON OF VIRGIN AND CULTIVATED SOILS

Since nearly as much phosphorus, potassium and calcium was found in solution in the short as in the longer acid extraction, the effect of similar

TABLE 1

*Plant-food elements extracted from air-dried soils when digested in 0.2 N $HNO_3$ for different periods of time*

| SOIL NUMBER | PHOSPHORUS | | | POTASSIUM | | | CALCIUM | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 minutes* | 5 minutes | 5 hours | 0 minutes | 5 minutes | 5 hours | 0 minutes | 5 minutes | 5 hours |
| | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* |
| 43506 | 8 | | 7 | 61 | | 114 | 1000 | | 1100 |
| 43526 | 10 | | 13 | 51 | | 63 | 540 | | 410 |
| 50117 | 19 | | 16 | 52 | | 63 | 240 | | 460 |
| 56527‡ | 10 | 12 | 10 | 105 | 122 | 138 | 1680 | 1880 | 1870 |
| 56528§ | 25 | | 22 | 79 | | 98 | 790 | | 840 |
| 56529‡ | 9 | 12 | 8 | 88 | 99 | 118 | 510 | 490 | 510 |
| 56530‡ | 12 | 14 | 14 | 113 | 132 | 154 | 970 | 1030 | 1080 |
| 56532‡ | 10 | 10 | 11 | 51 | 78 | 93 | 960 | 1050 | 1140 |
| 56583 | 34 | | 43 | 104 | | 137 | 470 | | 540 |
| 56584 | 16 | 15 | 16 | 35 | 42 | 43 | 560 | 520 | 580 |
| 56585 | 16 | 30 | 18 | 56 | 84 | 95 | 790 | 990 | 1020 |
| 56586 | 17 | 18 | 12 | 105 | 153 | 183 | 460 | 590 | 630 |
| 56587 | 14 | 12 | 13 | 80 | 119 | 124 | 480 | 580 | 620 |
| 56588 | 21 | 25 | 19 | 88 | 100 | 114 | 890 | 910 | 1020 |
| 56592 | 13 | 10 | 9 | 96 | 117 | 135 | 750 | 790 | 800 |
| 56652 | 9 | | 5 | 115 | | 121 | 740 | | 670 |
| 56654 | 11 | | 5 | 37 | | 44 | 540 | | - |
| Average†..... | 14 | 16 | 13 | 82 | 105 | 120 | 805 | 883 | 927 |
| Relative solubility..... | *108* | *123* | *100* | *77* | *88* | *100* | *87* | *95* | *100* |

* See p. 385.

† Only those samples are included in which digestions were made for the three periods.

‡ 100 gm. of soil taken for the digestion.

§ 110 gm. of soil taken for the digestion.

digestions on the soil silicates was determined. Several comparisons have also been made of virgin with the corresponding cultivated soil. The results are given in table 2.

### EFFECT OF TOBACCO CULTIVATION ON SOME SOIL CONSTITUENTS

It is generally believed in this locality that tobacco rapidly impoverishes the soil, possibly due to the fact that a comparatively large yield is obtained

on good land and this from a plant with shallow roots which necessarily derives most of its sustenance from the surface layer. Moreover it has a very high ash and withdraws relatively large amounts of potassium, nitrogen and calcium from the soil. For this reason it is generally grown on virgin or old sod land and after two or three crops have been removed the land is put in clover or grass to restore its fertility. Some digestions were made of such soils and also of the corresponding virgin samples. Most of the soils worked upon contain considerable calcium phosphate which is naturally unevenly distributed. As this material is gradually soluble in 0.2 *N* $HNO_3$, this probably accounts for some irregularities and high figures obtained for phosphorus and calcium, especially in the 5-hour extraction. The results are given in table 3.

### EFFECT OF AIR DRYING THE SOIL AND THE PRESENCE OF LIMESTONE IN SAME ON THE 0.2 *N* $HNO_3$ DIGESTION

Some experiments have been made in determining what effect digesting the moist soil in 0.2 *N* $HNO_3$ without preliminary air-drying would have on the results obtained as compared with the air-dried sample. Experiments have also been made on testing the effect produced by limestone on the solubility of plant-food constituents when it has been in contact with the moist soil some time previous to the acid digestion. In all of these experiments, the moisture content of the soil was maintained at 20 per cent for several weeks previous to the acid extraction. Moist soil equivalent to the usual quantity of the moisture free sample employed was used for the digestion. In the moist soils which contained limestone stronger acid was employed as explained under "Methods." The water present in the moist soil was also considered in the preparation of the solvent so that the latter would not be diluted in the extraction. The limestone used was practically pure $CaCO_3$ and was 100-mesh fine. It was thoroughly mixed with the soil at the rate of 4000 parts per million of soil. The required amount of water was then added to maintain the above moisture content. The results are given in table 4 together with those obtained on the air dried samples recorded in tables 1 and 2.

### EFFECT OF VARIABLE AMOUNTS OF SOIL IN THE ACID DIGESTION

Some of the foregoing experiments indicate that adsorption exerts a considerable influence on the results obtained. If this is true, then it may be influenced by the character and possibly to some extent by the quantity of soil used as well as by the duration of the extraction. In order to determine if quantity was a factor, experiments have been made in digesting different amounts of the same soil in their proportionate volumes of acid. The results are given in table 5.

TABLE 2

*Amounts of plant-food elements extracted from air-dried soils when digested in 0.2 N $HNO_3$ for different periods of time*

| SOIL NUMBER | CHARACTER | PHOSPHORUS | | POTASSIUM | | CALCIUM | | SILICON | |
|---|---|---|---|---|---|---|---|---|---|
| | | 5 minutes | 5 hours | 5 minutes | 5 hours | 5 minutes | 5 hours | 5 minutes | 5 hours |
| | | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* |
| 2305 | Cult. 9 years. | 153 | 145 | 138 | 157 | 3640 | 3730 | 63 | 237 |
| 2306 | Cult. 33 years. | 25 | 23 | 101 | 117 | 2580 | 2570 | 53 | 191 |
| 9768 | Virgin | 62 | 62 | 206 | 219 | 6050 | 6500 | 90 | 279 |
| 9771 | Cult. | 15 | 19 | 80 | 92 | 920 | 940 | 22 | 93 |
| 14411 | Cult. | 32 | 36 | 153 | 170 | 3220 | 3240 | 33 | 153 |
| 14412 | Virgin | 146 | 191 | 451 | 496 | 4420 | 4860 | 58 | 242 |
| 17483 | Cult. | 126 | 158 | 144 | 208 | 2290 | 2680 | 32 | 155 |
| 17485 | Virgin | 113 | 122 | 206 | 188 | 1780 | 1930 | 33 | 145 |
| 25002 | Cult. | 4 | 8 | 124 | 120 | 790 | 1020 | 36 | 106 |
| 25004 | Virgin | 10 | 15 | 200 | 222 | 1290 | 1350 | 34 | 88 |
| 25662 | Cult. | 13 | 9 | 132 | 150 | 710 | 760 | 22 | 84 |
| 25663 | Virgin | 21 | 15 | 102 | 132 | 380 | 400 | 24 | 82 |
| 25796 | Cult. | 27 | 31 | 92 | 105 | 1690 | 1770 | 46 | 177 |
| 25797 | Virgin | 55 | 74 | 202 | 227 | 1920 | 2070 | 62 | 220 |
| 36263 | Virgin | 9 | 9 | 77 | 84 | 310 | 280 | 14 | 53 |
| 36538 | Virgin | 27 | 26 | 205 | 230 | 3400 | 3560 | 70 | 118 |
| 36539 | Cult. | 17 | 11 | 185 | 207 | 1630 | 1700 | 61 | 99 |
| 36694 | Cult. | 28 | 43 | 64 | 74 | 790 | 860 | 18 | 85 |
| 36696 | Virgin | 42 | 51 | 149 | 162 | 1330 | 1410 | 33 | 139 |
| 36792 | Virgin | 27 | 28 | 106 | 118 | 880 | 870 | 32 | 130 |
| 36796 | Cult. | 23 | 17 | 87 | 93 | 500 | 510 | 24 | 83 |
| 56447 | Cult. | 8 | 10 | 156 | 174 | 650 | 690 | 18 | 51 |
| 54449 | Virgin | 20 | 21 | 250 | 256 | 3030 | 3140 | 47 | 108 |
| 56489 | Virgin‡ | 4 | 6 | 124 | 137 | 1020 | 1080 | 25 | 98 |
| 56490 | Cult.‡ | 4 | 6 | 106 | 117 | 820 | 840 | 41 | 116 |
| 56491 | Virgin‡ | 3 | 6 | 126 | 140 | 650 | 720 | 28 | 79 |
| 56492 | Cult.‡ | 4 | 6 | 61 | 72 | 1090 | 1130 | 36 | 101 |
| 56493 | Virgin | 11 | 14 | 225 | 256 | 2830 | 2710 | 41 | 137 |
| 56495 | Cult. | 5 | 8 | 68 | 72 | 820 | 850 | 16 | 84 |
| 56497 | Cult. | 14 | 15 | 84 | 99 | 730 | 750 | 24 | 84 |
| 56499 | Virgin | 20 | 23 | 226 | 244 | 2090 | 2110 | 36 | 130 |
| 56501 | Cult. | 84 | 109 | 82 | 86 | 3980 | 3770 | 56 | 382 |
| 56503 | Virgin | 104 | 125 | 95 | 104 | 4600 | 4710 | 68 | 383 |
| 56525 | Cult. | 7 | 7 | 86 | 63 | 300 | 340 | 12 | 26 |
| 56526 | Cult. | 19 | 22 | 153 | 174 | 1140 | 1230 | 49 | 220 |
| 56531† | Cult. | 12 | 13 | 97 | 69 | 260 | 280 | 38 | 73 |
| 56534 | Cult. | 4396 | 4816 | 70 | 97 | 13810 | 16020 | | |
| 56549 | Cult. | 19 | 28 | 68 | 79 | 830 | 780 | 29 | 107 |
| 56699 | Cult. | 6 | 11 | 91 | 103 | 2000 | 2050 | 86 | 277 |
| 56700 | Cult. | 17 | 21 | 118 | 135 | 1010 | 1000 | 36 | 160 |

TABLE 2—*Continued*

| SOIL NUMBER | CHARACTER | PHOSPHORUS | | POTASSIUM | | CALCIUM | | SILICON | |
|---|---|---|---|---|---|---|---|---|---|
| | | 5 minutes | 5 hours | 5 minutes | 5 hours | 5 minutes | 5 hours | 5 minutes | 5 hours |
| | | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* |
| 56701 | Cult. | 6 | 6 | 107 | 119 | 1470 | 1290 | 141 | 182 |
| 56702 | Cult. | 11 | 14 | 48 | 53 | 420 | 380 | 26 | 127 |
| 56727 | Cult. | 23 | 28 | 120 | 142 | 2380 | 2500 | 86 | 392 |
| 56728 | Cult. | 79 | 83 | 148 | 178 | 3610 | 3770 | 189 | 625 |
| 56733 | Cult. | 51 | 61 | 82 | 87 | 1090 | 1160 | | |
| 56740 | Cult. | 5 | 6 | 58 | 68 | 1530 | 1720 | 70 | 213 |
| 56741 | Cult. | 6 | 7 | 87 | 96 | 660 | 750 | 26 | 139 |
| 56742 | Cult. | 8 | 10 | 104 | 125 | 1280 | 1350 | 29 | 153 |
| 61036 | Cult. | 5 | 9 | 54 | 58 | 720 | 800 | 23 | 127 |
| 61037 | Cult. | 5 | 9 | 58 | 67 | 890 | 930 | 24 | 100 |
| 61122 | Cult. | 5 | 12 | 91 | 105 | 1060 | 1100 | 23 | 134 |
| 61123 | Cult. | 6 | 10 | 60 | 68 | 580 | 600 | 13 | 93 |
| 61244 | Cult. | 4 | 9 | 108 | 123 | 690 | 660 | 32 | 89 |
| 61246 | Virgin | 5 | 8 | 106 | 116 | 250 | 230 | 10 | 44 |
| 61253 | Cult. | 5 | 8 | 128 | 140 | 350 | 350 | 19 | 68 |
| 61254 | Cult.§ | 4 | 7 | 108 | 113 | 310 | 320 | 55 | 172 |
| 61255 | Virgin | 6 | 9 | 87 | 92 | 100 | 200 | 18 | 43 |
| 61256 | Virgin§ | 5 | 6 | 97 | 100 | 170 | 180 | 34 | 123 |
| 61265 | Cult. | 4 | 7 | 63 | 69 | 790 | 820 | 20 | 105 |
| 61266 | Cult.§ | 7 | 9 | 66 | 70 | 420 | 420 | 38 | 124 |
| 61267 | Virgin | 3 | 8 | 63 | 66 | 310 | 310 | 22 | 91 |
| 61268 | Virgin§ | 2 | 7 | 69 | 66 | 130 | 160 | 38 | 146 |
| Average* | | 26 | 30 | 121 | 133 | 1442 | 1501 | 88 | 316 |
| *Relative solubility* | | *87* | *100* | *91* | *100* | *96* | *100* | *28* | *100* |

* Only those samples are included in which the silicon was also determined,

† 10 gm. of soil taken for the digestion.

‡ Sample of the first 18 inches.

§ Subsoil of the preceding soil at depth of 6–18 inches.

## DISCUSSION OF RESULTS AND CONCLUSIONS

There have been 92 soils used in this investigation, representing the different soil areas found in this state. The results show that there are 30 samples in which the percentage of phosphorus, 5 in which the percentage of potassium and 16 in which the percentage of calcium found by the short digestion equals or exceeds the percentages of these elements obtained by the longer extraction. The silicon obtained, however, by the short digestion is less in every sample. If a direct comparison be made of the figures obtained for each element where it was determined in the same sample, both by the 5 minute and 5 hour digestion, it will be found that there are 73 soils in which the silicon was determined and 85 soils in which only phosphorus, potassium and calcium were estimated. Taking the averages represented by these

samples, it will be found that 91 per cent of the phosphorus, 89 per cent of the potassium, 94 per cent of the calcium and only 28 per cent of the silicon are obtained in the short digestion as compared with the regular procedure. If the averages of all soils used are considered, the results are still better, namely, 97 per cent, 92 per cent, 99 per cent and 28 per cent, respectively, for the above elements.

The experiments indicate that adsorption in some soils is a factor which may appreciably affect the results obtained for certain plant-food constituents in the 0.2 *N* $HNO_3$ digestion as ordinarily employed. With such soils, there-

TABLE 3

*Effect of tobacco cultivation on the amounts of plant food elements extracted from air-dried soils when digested in 0.2 N $HNO_3$ for different periods of time*

| SOIL NUMBER | CHARACTER | PHOSPHOROUS | | POTASSIUM | | CALCIUM | | SILICON | |
|---|---|---|---|---|---|---|---|---|---|
| | | 5 minutes | 5 hours | 5 minutes | 5 hours | 5 minutes | 5 hours | 5 minute | 5 hours |
| | | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* |
| 56747 | Virgin | 954 | 780 | 180 | 222 | 27460 | 29370 | 181 | 394 |
| 56748 | Tobacco, 2 years. | 246 | 234 | 142 | 169 | 5840 | 6200 | 124 | 350 |
| 56959 | Cult. good crop* | 24 | 24 | 73 | 90 | 1910 | 2150 | 31 | 120 |
| 56960 | Cult. poor crop | 24 | 18 | 47 | 54 | 1780 | 1850 | 34 | 132 |
| 61192 | Cult. good crop† | 14 | 25 | 107 | 119 | 950 | 970 | 37 | 145 |
| 61193 | Cult. poor crop‡ | 8 | 8 | 24 | 32 | 720 | 770 | 28 | 117 |
| 61194 | Virgin | 129 | 148 | 225 | 258 | 2600 | 2760 | 36 | 170 |
| 61195 | Tobacco, 2 years | 112 | 165 | 247 | 288 | 2480 | 2530 | 33 | 159 |
| 61196 | Tobacco, 3 years | 166 | 239 | 106 | 130 | 1990 | 2480 | 23 | 142 |
| 61197 | Virgin | 296 | 370 | 264 | 303 | 3490 | 3900 | 44 | 214 |
| 61198 | Tobacco 2 years | 278 | 378 | 211 | 254 | 3530 | 3740 | 56 | 223 |
| 61199 | Virgin | 429 | 464 | 271 | 306 | 3950 | 4250 | 48 | 242 |
| 61200 | Tobacco, 2 years | 535 | 626 | 187 | 217 | 5340 | 6030 | 75 | 302 |
| Average | | 247 | 268 | 160 | 188 | 4772 | 5154 | 58 | 209 |
| *Relative solubility* | | *92* | *100* | *85* | *100* | *93* | *100* | *28* | *100* |

* This land was not in tobacco. The good crop was on land in cultivation 1 year while soil no. 56960, with the same soil characteristics, had been cultivated several years, part of the time in tobacco.

† From a field of corn where the growth was good.

‡ From the same field where the growth was poor.

fore, the result usually reported for the prescribed 5-hour period represents the quantity dissolved by the acid during this time less the amount subsequently withdrawn from solution by the soil during the same time due to adsorption. Assuming that the latter is not an instantaneous process, the less time that the soil is in contact with the acid would be advantageous in partly overcoming this effect. The short digestion is, therefore preferable on this account inasmuch as the averages obtained in this work show that it gives about as good results and moreover shortens the regular procedure.

TABLE 4

*Effect of air-drying and addition of limestone on the amounts of plant food elements extracted from moisture-free soils when digested in 0.2 N $HNO_3$*

| NUMBER | TREATMENT | PHOSPHORUS (DIGESTION = 0 MINUTES§) | POTASSIUM (DIGESTION = 0 MINUTES§) | CALCIUM (DIGESTION = 0 MINUTES§) |
|---|---|---|---|---|
| | | *p.p.m.* | *p.p.m.* | *p.p.m.* |
| 56584 | Moist | 19 | 40 | 570 |
| | Moist, limed | 18 | 27 | 2110 |
| | Air-dried | 16 | 36 | 570 |
| 56585 | Moist | 31 | 83 | 1030 |
| | Moist, limed | 32 | 70 | 2520 |
| | Air-dried | 16 | 57 | 800 |
| 56586 | Moist | 21 | 148 | 580 |
| | Moist* | 16 | 153 | 630 |
| | Moist, limed | 22 | 127 | 1880 |
| | Moist, limed* | 18 | 145 | 1990 |
| | Air-dried | 17 | 107 | 470 |
| 56587 | Moist | 15 | 116 | 660 |
| | Moist, limed | 18 | 111 | 2210 |
| | Air-dried | 14 | 81 | 490 |
| 56588 | Moist | 23 | 102 | 1010 |
| | Moist, limed | 26 | 91 | 2460 |
| | Air-dried | 21 | 89 | 900 |
| 56592 | Moist | 13 | 134 | 820 |
| | Moist* | 11 | 112 | 800 |
| | Moist, limed | 15 | 134 | 2400 |
| | Moist, limed* | 12 | 108 | 2170 |
| | Air-dried | 13 | 97 | 760 |
| 56652 | Moist | 9 | 98 | 760 |
| | Moist, limed | 13 | 116 | — |
| | Air-dried | 9 | 117 | 750 |
| 56654 | Moist | 9 | 25 | 510 |
| | Moist, limed | 10 | 11 | — |
| | Air-dried | 11 | 38 | 550 |
| 56699 | Moist, limed* | 6 | 86 | 2020 |
| | Air-dried, limed† | 6 | 92 | 2030 |
| 56700 | Moist* | 16 | 116 | 1000 |
| | Air-dried† | 17 | 120 | 1030 |
| 56701 | Moist* | 7 | 100 | 1340 |
| | Air-dried† | 6 | 109 | 1490 |
| 56702 | Moist* | 11 | 48 | 420 |
| | Air-dried† | 11 | 49 | 430 |
| 56740 | Air-dried† | 5 | | 1550 |
| | Moist, limed‡ | 7 | | 1640 |
| 56741 | Air-dried† | 6 | | 670 |
| | Moist‡ | 6 | | 760 |
| 56742 | Air-dried† | 8 | | 1300 |
| | Moist‡ | 8 | | 1420 |

* Digested for 5 minutes.

† Digested soil for 5 minutes.

‡ Digested amount equivalent to 50 gm. air-dried soil in 500 cc. of 0.2 *N* $HNO_3$. This was one-half of usual quantities of soil and acid employed.

§ See page 385.

TABLE 5

*Effect of variable amounts of soil on the quantities of plant-food elements extracted when digested in 0.2 N $HNO_3$*

| SOIL NUMBER | AMOUNT OF SOIL USED§ | PHOSPHORUS (DIGESTION = 5 MINUTES) | POTASSIUM (DIGESTION = 5 HOURS) | CALCIUM (DIGESTION = 5 MINUTES) |
|---|---|---|---|---|
| | *gm.* | *p.p.m.* | *p.p.m.* | *p.p.m.* |
| 43506 | 120 | .... | 114 | |
| | 50 | .... | 89 | |
| 43526 | 120 | .... | 63 | |
| | 50 | .... | 74 | |
| 50117 | 120 | .... | 63 | |
| | 50 | .... | 72 | |
| 56525 | 120 | 7 | 63 | 300 |
| | 50 | 10 | 95 | 350 |
| 56526 | 120 | 19 | 174 | 1140 |
| | 50 | 40 | 167 | 1430 |
| 56527 | 100 | 12 | 138 | 1880 |
| | 50 | 14 | 164 | 1960 |
| 56528 | 110 | 25b | 98 | 790* |
| | 50 | 33b | 101 | 980* |
| 56529 | 100 | 12 | 118 | 490 |
| | 50 | 12 | 114 | 620 |
| 56530 | 100 | 14 | ... | 1030 |
| | 50 | 9 | ... | 1100 |
| 56531 | 110 | 12 | ... | 260 |
| | 50 | 15 | ... | 360 |
| 56532 | 100 | 10 | 93 | 1050 |
| | 50 | 5 | 64 | 1110 |
| 56584 | 120 | .... | 43 | |
| | 50 | .... | 46 | |
| 56586 | 120 | .... | 183 | |
| | 50 | .... | 167 | |
| 56592 | 120 | .... | 135 | |
| | 50 | .... | 169 | |
| 56700 | 120 | .... | 135 | |
| | 50 | .... | 132 | |
| 56702 | 120 | .... | 53 | |
| | 50 | .... | 59 | |
| 56740 | 120 | 5 | ... | 1530 |
| | 50 | 7 | ... | 1640 |
| 56741 | 120 | 6 | ... | 660 |
| | 50 | 6 | ... | 760 |
| 56742 | 120 | 8 | ... | 1280 |
| | 50 | 8 | ... | 1420 |
| 56747 | 120 | 780† | 222 | 29370† |
| | 50 | 744† | 216 | 29320† |
| 56748 | 120 | 234† | 169 | 6200† |
| | 50 | 196† | 164 | 6110† |

§ The relative proportions used were 1 gm. soil to 10 cc. of 0.2 *N* $HNO_3$

* Digestion = 0 minutes, see p. 385.

† Digestion = 5 hours.

It will probably be conceded that the solvent used in any method of this character should have very little action on the soil silicates. The fact that they are not attacked to the same extent in the short digestion as shown for the silicon in tables 2 and 3 while the bulk of the other elements are obtained makes this procedure look more favorable for the purpose intended.

That the weak acid digestion as employed here does have some value for purposes of comparison at least for indicating possible deficiencies of plant food elements in soils is shown in the same tables. In making comparisons by this method, however, it is essential that the extractions be carried on together and on the same quantity of soil. This latter point is demonstrated in table 5.

The addition of calcium carbonate to the soil as shown in table 4 increases the amount of phosphorus and decreases the amount of potassium found in the 0.2 *N* $HNO_3$ extract of most of these samples. On the other hand, failure to dry the sample for the acid digestion appears to exert a variable influence on the amount of the above elements found in solution as compared with a similar digestion of the air dired soil. For instance, in table 4, among the soils worked upon, there are some where the amounts of both of these elements are increased when the moist soil was used but on the contrary there are about as many which show either no effect or a loss. For this reason it is difficult to draw general conclusions regarding this factor as this seems to depend on the character of the soil.

Notwithstanding that it is an empirical procedure, the writer believes that the weak acid digestion, especially of short duration as employed in this investigation is valuable for indicating possible deficiencies of plant food elements in soils, provided it is used for purposes of comparison or in connection with a soil survey.

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# VARIABILITY OF NITRATES AND TOTAL NITROGEN IN SOILS[1]

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The reliability of chemical determinations on soils, in their application to large or small areas of land of apparent uniformity, has been given much consideration by investigators recently. It is well recognized that a large plot of land, even if the soil were apparently uniform in appearance, would require a large number of samples, composited and analyzed separately in order to give a reliable index for the whole area. Waynick (8) and Waynick and Sharp (9) have called attention to the great variability of nitrates and also the extreme variation in nitrogen and carbon content in field samples of soil.

In the plot work at the New Jersey Experiment Station, there has been a question in the mind of the writer as to how many samples should be taken for chemical work in order to secure results representative of the entire plot. The plots cover a relatively small area, ($\frac{1}{20}$ of an acre), and it would seem that a few samples, carefully taken and covering the plot should, when composited, fairly represent the area. An attempt has been made to study this problem by determining the nitrates and total nitrogen content of a large number of samples from three plots which have received different fertilizer treatment for the past 15 years. In studies on the same plots, Waksman (7) has recently shown the importance which the numbers of microörganisms play in indicating the fertility of a soil. Probably in the majority of cases nitrates would tend to be present in largest amounts in the more fertile soils. However, Blair and Prince (2) have shown that nitrates may be formed in considerable quantities in a soil that is so acid as to practically inhibit growth of ordinary farm crops. An opportunity was offered in the present work to compare soils under different fertilizer treatment with reference to their nitrate content and microbial flora.

## METHODS

The plots selected for this work belong to the series at the New Jersey Agricultural Experiment Station, which is being used in a study of the availability of nitrogenous fertilizers. The plots are one-twentieth of an acre in size and were laid out in 1908. Since then they have been under the same system of fertilization and cropping. The soil is a Sassafras loam. The

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[2] The writer is indebted to Dr. S. A. Waksman for suggesting the problem and for valuable suggestions during the progress of the work.

rotation is a general farm crop rotation—corn, oats, wheat, and 2 years of timothy. The details of the experiment together with the chemical analyses of the soil and crops are reported in earlier papers by Lipman and Blair (3, 4). The particular plots chosen for this work were 5A, 7A, and 9A. These plots have received no lime during the period. The fertilizer treatment on the acre basis for these plots has been as follows: plot 5A, 640 pounds acid phosphate, 320 pounds potassium chloride and 16 tons of cow manure annually; plot 7A, nothing; plot 9A, 640 pounds acid phosphate, 320 pounds potassium chloride and 320 pounds sodium nitrate annually. The total yield of dry matter (hay, straw, grain) for the past 15 years has been 70,191 pounds for plot 5A; 15,774 pounds for plot 7A; and 57,848 pounds for plot 9A.

Twenty-five samples were taken from each plot during August, 1922 and distributed as shown in figure 1. The plots are 65.87 feet by 33.36 feet wide so that each sample was about 8–10 feet from those adjacent. In taking samples a tube cutting a core about one inch in diameter was used. Three such borings to the depth of 6⅔ inches were taken within a radius of 5–6 inches for each sample. The soil was quite moist, although it had not rained for about one week. The samples were brought to the laboratory in covered pint jars. The following day they were passed through a 5-mm. sieve and thoroughly mixed. There was only a very small amount of detritus (10–20 gm). In order to study not only the variation between the samples, but also to learn if there was a correlation between nitrates and the nitrifying capacity of the soil, all the samples were tested for their nitrifying power. One hundred grams were tested for their nitrifying power. One hundred grams of the soil from each of the samples was weighed into tumblers. Thirty milligrams of nitrogen in the form of ammonium sulfate was used as a source of nitrogen. It is known that $(NH_4)_2SO_4$ tends to make a soil acid, and consequently the microbial flora would be affected and nitrification processes interfered with. In order to prevent, as nearly as possible, any change in the original acidity of the soils, 0.2 gm. calcium carbonate was added to each tumbler to neutralize the acids which would be formed from the addition of ammonium sulfate. The soil in the tumblers was stirred thoroughly and allowed to incubate at 28°C. for 28 days, keeping it at an optimum moisture content. Moisture determinations were run on several of the original samples from each plot. Plot 5A averaged 11.5 per cent water; plot 7A, 9.5 per cent and plot 9A, 12.5 per cent water.

The remainder of the soil from the original samples was air-dried and passed through a 2-mm. sieve. Nitrate determinations were made on each sample by the phenoldisulfonic acid method (5). At the expiration of 28 days, the soils in the nitrification experiments were spread out and allowed to dry, then passed through a 2-mm. sieve and the nitrates determined as above. The results for the nitrate determinations on the untreated soils are given in table 1 and for the nitrification studies, in table 2. A composite sample was made by taking 25 gm. from each individual sample. This was mixed thoroughly and ten determinations for nitrates made on each composite. The results are given in table 3.

The mean, coefficient of variability, and probable error of the mean have been calculated for each plot. The methods of calculation were those employed by Waynick (8) and also by Waksman (6). The standard deviation, which is represented by $\delta$ is found by squaring the deviation from the mean, taking the sum of the squares thus found, dividing this figure by the total number of determinations and taking the square root of the quotient. The coefficient variability *C. V.* is simply the percentage ratio of the standard deviation to the mean. The probable error of the mean *Em* is given by the formula:

$$Em = \frac{0.6745 \times \delta}{\sqrt{N}}$$

where N stands for the number of determinations.

## DISCUSSION OF TABLES 1, 2, AND 3

The most striking observation to be noticed from table 1 is the great variation in the nitrate content existing between the various samples within each plot. The coefficient of variability for plot 5A is 34.7 per cent, which

means that approximately two-thirds of the determinations may be expected to lie within that range on either side of the mean. That is, the range within which two-thirds of the determinations may be expected to fall is 0.31–0.67 milligrams in this case. The extreme range would of course be much greater.

TABLE 1

*Nitrate in soil as sampled*

| SAMPLE NUMBER | PLOT 5A | | PLOT 7A | | PLOT 9A | |
|---|---|---|---|---|---|---|
| | Nitrate nitrogen | Deviation from mean ± | Nitrate nitrogen | Deviation from mean ± | Nitrate nitrogen | Deviation from mean ± |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 1 | 0.41 | 0.08 | 0.16 | 0.01 | 0.32 | 0.00 |
| 2 | 0.56 | 0.07 | 0.13 | 0.04 | 0.36 | 0.04 |
| 3 | 0.51 | 0.02 | 0.15 | 0.02 | 0.35 | 0.03 |
| 4 | 0.62 | 0.13 | 0.17 | 0.00 | 0.43 | 0.11 |
| 5 | 0.51 | 0.02 | 0.13 | 0.04 | 0.42 | 0.10 |
| 6 | 0.67 | 0.18 | 0.14 | 0.03 | 0.43 | 0.11 |
| 7 | 0.53 | 0.04 | 0.15 | 0.02 | 0.34 | 0.02 |
| 8 | 0.54 | 0.05 | 0.17 | 0.00 | 0.45 | 0.13 |
| 9 | 0.26 | 0.23 | 0.15 | 0.02 | 0.34 | 0.02 |
| 10 | 0.26 | 0.23 | 0.17 | 0.00 | 0.44 | 0.12 |
| 11 | 0.29 | 0.20 | 0.25 | 0.08 | 0.42 | 0.10 |
| 12 | 0.19 | 0.30 | 0.19 | 0.02 | 0.32 | 0.00 |
| 13 | 0.64 | 0.15 | 0.15 | 0.02 | 0.34 | 0.02 |
| 14 | 0.61 | 0.12 | 0.14 | 0.03 | 0.34 | 0.02 |
| 15 | 0.66 | 0.17 | 0.21 | 0.04 | 0.19 | 0.13 |
| 16 | 0.58 | 0.09 | 0.16 | 0.01 | 0.34 | 0.02 |
| 17 | 0.28 | 0.21 | 0.15 | 0.02 | 0.19 | 0.13 |
| 18 | 0.58 | 0.09 | 0.17 | 0.00 | 0.24 | 0.08 |
| 19 | 0.46 | 0.03 | 0.17 | 0.00 | 0.25 | 0.07 |
| 20 | 0.51 | 0.02 | 0.16 | 0.01 | 0.24 | 0.08 |
| 21 | 0.66 | 0.17 | 0.19 | 0.02 | 0.16 | 0.16 |
| 22 | 0.96 | 0.47 | 0.22 | 0.05 | 0.45 | 0.13 |
| 23 | 0.30 | 0.19 | 0.19 | 0.02 | 0.18 | 0.14 |
| 24 | 0.27 | 0.22 | 0.25 | 0.08 | 0.34 | 0.02 |
| 25 | 0.30 | 0.19 | 0.17 | 0.00 | 0.29 | 0.03 |
| Mean.... | 0.49 mgm. | 0.15 mgm. | 0.17 mgm. | 0.02 mgm. | 0.32 mgm. | 0.07 mgm. |
| $\sigma$..... | 0.18 | | 0.03 | | 0.09 | |
| *C.V*..... | 34.7% | | 17.7% | | 28.1% | |
| *Em*..... | 0.024 mgm. or 4.95% | | 0.004 mgm. or 2.38% | | 0.012 mgm. or 3.80% | |

The coefficient of variability for plot 7A is 17.7 per cent and for 9A, it is 28 per cent. Since plot 5A has been highly manured, it would be expected to show the greatest variation in nitrate content as, in reality, it does; while plot 7A which has received no fertilizer treatment, is the least variable. The variation is also great in 9A where sodium nitrate had been applied. The probable errors of the mean in each case vary in the same proportion.

Table 2 which records data on the nitrification studies also shows much variation among the individual samples, but the coefficient of variability and probable errors of the mean on plot 5A and 9A are much reduced, while the variation between samples on 7A is increased immensely. This would indicate

TABLE 2

*Nitrate produced in 100 gm. of soil from ammonium sulfate in 28 days*

| SAMPLE NUMBER | PLOT 5A | | PLOT 7A | | PLOT 9A | |
|---|---|---|---|---|---|---|
| | Nitrate nitrogen | Deviation from mean ± | Nitrate nitrogen | Deviation from mean ± | Nitrate nitrogen | Deviation from mean ± |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 1 | 24.0 | 0.2 | 2.4 | 1.8 | 11.6 | 5.8 |
| 2 | 26.4 | 2.6 | 3.2 | 1.0 | 19.2 | 1.8 |
| 3 | 27.2 | 3.4 | 3.4 | 0.8 | 18.8 | 1.4 |
| 4 | 27.2 | 3.4 | 2.5 | 1.7 | 16.0 | 1.4 |
| 5 | 28.0 | 4.2 | 2.2 | 2.0 | 13.6 | 3.8 |
| 6 | 28.0 | 4.2 | 3.0 | 1.2 | 18.0 | 1.4 |
| 7 | 27.2 | 3.4 | 2.6 | 1.6 | 17.6 | 0.2 |
| 8 | 25.6 | 1.8 | 4.5 | 0.3 | 20.0 | 2.6 |
| 9 | 24.8 | 1.0 | 2.2 | 2.0 | 19.2 | 1.8 |
| 10 | 25.6 | 1.8 | 3.4 | 0.8 | 19.6 | 2.2 |
| 11 | 25.6 | 1.8 | 3.4 | 0.8 | 20.0 | 2.6 |
| 12 | 25.6 | 1.8 | 3.6 | 0.6 | 22.0 | 4.6 |
| 13 | 23.2 | 0.6 | 4.0 | 0.2 | 22.4 | 5.0 |
| 14 | 24.8 | 1.0 | 2.8 | 1.4 | 14.8 | 2.6 |
| 15 | 24.8 | 1.0 | 4.3 | 0.1 | 16.8 | 0.6 |
| 16 | 24.0 | 0.2 | 3.7 | 0.5 | 16.0 | 1.4 |
| 17 | 24.0 | 0.2 | 4.2 | 0.0 | 19.6 | 2.2 |
| 18 | 23.2 | 0.6 | 5.0 | 0.8 | 19.2 | 1.8 |
| 19 | 19.2 | 4.6 | 5.4 | 1.2 | 17.6 | 0.2 |
| 20 | 21.8 | 2.0 | 3.6 | 0.6 | 12.0 | 5.4 |
| 21 | 20.8 | 3.0 | 5.6 | 1.4 | 13.2 | 4.2 |
| 22 | 21.3 | 2.5 | 5.7 | 1.5 | 17.6 | 0.2 |
| 23 | 18.6 | 5.2 | 8.0 | 3.8 | 15.6 | 1.8 |
| 24 | 17.0 | 6.8 | 8.4 | 4.2 | 16.0 | 1.4 |
| 25 | 17.0 | 6.8 | 8.2 | 4.0 | 19.2 | 1.8 |
| Mean.... | 23.8 mgm. | 2.6 mgm. | 4.2 mgm. | 1.4 mgm. | 17.4 mgm. | 2.3 mgm. |
| $\sigma$..... | 3.19 | | 1.77 | | 2.82 | |
| *C.V.*..... | 13.4% | | 42.1% | | 16.2% | |
| *Em*..... | 0.431 mgm. or 1.81% | | 0.239 mgm. or 5.68% | | 0.380 mgm. or 2.18% | |

that certain sections of plot 7A are capable of facilitating nitrification processes more rapidly than others. The upper half of the plot (samples 15–25) gave by far the highest yields of nitrates on this plot. It will also be noted in table 1 that samples 13, 14, 15, and 16 which lie in the center of the plot, yielded very high nitrates for plot 5A. These samples lie in a straight line

across the plot and no doubt indicate that there was an accumulation of manure and microörganisms in this section. This may have been due to ploughing which was done in this direction.

In table 3, the nitrates are given for the composite sample made from the 25 individual samples. In all cases the mean runs considerably lower than the mean for the 25 individual samples. It is difficult to explain this discrepancy, unless it is due to the fact that the determinations on the composite samples were not made until a month later. However, as all the samples

TABLE 3

*Nitrate in soil-composite of twenty-five samples*

| SAMPLE NUMBER | PLOT 5A | | PLOT 7A | | PLOT 9A | |
|---|---|---|---|---|---|---|
| | Nitrate nitrogen | Deviation from mean ± | Nitrate nitrogen | Deviation from mean ± | Nitrate nitrogen | Deviation from mean ± |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 1 | 0.34 | 0.03 | 0.12 | 0.01 | 0.25 | 0.01 |
| 2 | 0.36 | 0.01 | 0.12 | 0.01 | 0.24 | 0.00 |
| 3 | 0.37 | 0.00 | 0.11 | 0.00 | 0.21 | 0.03 |
| 4 | 0.37 | 0.00 | 0.12 | 0.01 | 0.25 | 0.01 |
| 5 | 0.37 | 0.00 | 0.10 | 0.01 | 0.24 | 0.00 |
| 6 | 0.38 | 0.01 | 0.10 | 0.01 | 0.22 | 0.02 |
| 7 | 0.40 | 0.03 | 0.11 | 0.00 | 0.25 | 0.01 |
| 8 | 0.35 | 0.02 | 0.10 | 0.01 | 0.26 | 0.02 |
| 9 | 0.35 | 0.02 | 0.12 | 0.01 | 0.23 | 0.01 |
| 10 | 0.36 | 0.01 | 0.12 | 0.01 | 0.23 | 0.01 |
| Mean.... | 0.365 mgm. | 0.013 mgm. | 0.11 mgm. | 0.008 mgm. | 0.24 mgm. | 0.012 mgm. |
| $\sigma$..... | 0.017 | | 0.0089 | | 0.0148 | |
| *C.V.*..... | 4.66% | | 8.09% | | 6.17% | |
| *Em*..... | 0.0036 mgm. or 1.0% | | 0.0019 mgm. or 1.72% | | 0.0032 mgm. or 1.31% | |

were dried at the same time, this should make no difference. The coefficient of variability and probable error of the mean on the composite samples are relatively low and indicate that the number of determinations on a well composited sample need be but few.

In order to study the data further, table 4 has been prepared. Here the mean of the ten lowest and ten highest nitrate determinations on the original soil is compared with the corresponding values for the same samples incubated with ammonium sulfate. The lowest and highest individual values and the mean of the twenty-five samples from each plot are also recorded. From the table it is clear that the original samples which are lowest in nitrate nitrogen also show the lowest nitrifying power while the original samples which are highest in nitrate nitrogen show a tendency toward greater nitrifying ability.

## TOTAL NITROGEN DETERMINATIONS

The second part of the work consisted in studying the variability of the total nitrogen content of the individual samples from each plot. The soils were passed through a 1-mm. sieve and the total nitrogen determined in duplicate on each individual sample by the ordinary Kjeldahl method (1). Ten determinations were also run on the composite sample from each plot made from the twenty-five individual samples. The data are given in tables 5 and 6. The coefficient of variability on all the plots is comparatively low for this determination, being not much over 5 per cent and the probable error of the mean is in all cases less than 1 per cent. The mean values are, for the three plots, decidedly different and correspond to their relative productivity.

TABLE 4

*Comparison of the mean values for the ten lowest and highest soil nitrates and for the corresponding samples treated with* $(NH_4)_2SO_4$

| | PLOT 5A | | PLOT 7A | | PLOT 9A | |
|---|---|---|---|---|---|---|
| | Nitrate nitrogen in untreated soil | Nitrate nitrogen in $(NH_4)_2SO_4$ —treated soil | Nitrate nitrogen in untreated soil | Nitrate nitrogen in $(NH_4)_2SO_4$ —treated soil | Nitrate nitrogen in untreated soil | Nitrate nitrogen in $(NH_4)_2SO_4$ —tseated soil |
| | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Mean of 25 samples | 0.49 | 23.8 | 0.17 | 4.2 | 0.32 | 17.4 |
| Mean of 10 lowest nitrate samples | 0.30 | 22.1 | 0.15 | 3.0 | 0.25 | 16.2 |
| Mean of 10 highest nitrate samples | 0.65 | 26.4 | 0.21 | 5.0 | 0.40 | 18.0 |
| Lowest individual value | 0.19 | 17.0 | 0.13 | 2.2 | 0.16 | 11.6 |
| Highest individual value | 0.96 | 28.0 | 0.25 | 8.4 | 0.45 | 22.4 |

The chief point to note in the discussion of this phase of the work is that the variability of total nitrogen content of soils is not nearly as great as in the case of the nitrates. One reason for this is the fact that the method of determining total nitrogen is not nearly as sensitive as the method for the determination of nitrates and the field errors of sampling are, consequently minimized. In the ordinary methods for the determinations of phosphorus and potassium, the same general tendency would probably hold true. That is, these methods are not sufficiently delicate to cause appreciable variability among a large number of samples taken from a small area, such as occurs, in the determination of nitrates. It would seem, therefore, quite unnecessary in determinations of this sort to have such a large number of samples. It is interesting to note from table 6 how close the mean values are for the composite samples as compared with the mean values for the twenty-five individual samples. In each case the difference is only one in the third place. The table also shows that it is unnecessary to run more than three determinations for nitrogen on a sample well composited.

It has been the customary practice in taking samples for chemical work from the experimental plots at this station to make a composite of about 9–12

individual samples covering the plot. In order to get an approximate idea of how truly representative such a sample would be, the mean of nine such samples covering the plot has been taken and compared with the mean of the total twenty-five samples. The nine chosen were samples 1, 4, 7, 9, 12, 17,

TABLE 5

*Total nitrogen in individual soils samples*

| SAMPLE NUMBER | PLOT 5A | | PLOT 7A | | PLOT 9A | |
|---|---|---|---|---|---|---|
| | Nitrogen content | Deviation from mean ± | Nitrogen content | Deviation from mean ± | Nitrogen content | Deviation from mean ± |
| | *gm. per 100* | *gm. per 100* | *gm. per 100* | *gm. per 100* | *gm. per 100* | *gm. per 100* |
| 1 | 0.143 | 0.005 | 0.070 | 0.006 | 0.093 | 0.011 |
| 2 | 0.139 | 0.001 | 0.071 | 0.005 | 0.099 | 0.005 |
| 3 | 0.136 | 0.002 | 0.074 | 0.002 | 0.108 | 0.004 |
| 4 | 0.126 | 0.012 | 0.076 | 0.000 | 0.103 | 0.001 |
| 5 | 0.132 | 0.006 | 0.072 | 0.004 | 0.094 | 0.010 |
| 6 | 0.138 | 0.000 | 0.073 | 0.003 | 0.103 | 0.001 |
| 7 | 0.138 | 0.000 | 0.073 | 0.003 | 0.108 | 0.004 |
| 8 | 0.130 | 0.008 | 0.080 | 0.004 | 0.104 | 0.000 |
| 9 | 0.135 | 0.003 | 0.075 | 0.001 | 0.107 | 0.003 |
| 10 | 0.147 | 0.009 | 0.072 | 0.004 | 0.101 | 0.003 |
| 11 | 0.137 | 0.001 | 0.082 | 0.006 | 0.115 | 0.011 |
| 12 | 0.135 | 0.003 | 0.084 | 0.008 | 0.113 | 0.009 |
| 13 | 0.148 | 0.010 | 0.076 | 0.000 | 0.100 | 0.004 |
| 14 | 0.154 | 0.016 | 0.070 | 0.006 | 0.102 | 0.002 |
| 15 | 0.136 | 0.002 | 0.071 | 0.005 | 0.110 | 0.006 |
| 16 | 0.130 | 0.008 | 0.080 | 0.004 | 0.110 | 0.006 |
| 17 | 0.138 | 0.000 | 0.082 | 0.006 | 0.098 | 0.006 |
| 18 | 0.142 | 0.004 | 0.072 | 0.004 | 0.101 | 0.003 |
| 19 | 0.135 | 0.003 | 0.076 | 0.000 | 0.103 | 0.001 |
| 20 | 0.139 | 0.001 | 0.080 | 0.004 | 0.107 | 0.003 |
| 21 | 0.143 | 0.005 | 0.081 | 0.005 | 0.096 | 0.008 |
| 22 | 0.159 | 0.021 | 0.075 | 0.001 | 0.096 | 0.008 |
| 23 | 0.138 | 0.000 | 0.076 | 0.000 | 0.102 | 0.002 |
| 24 | 0.142 | 0.004 | 0.080 | 0.004 | 0.106 | 0.002 |
| 25 | 0.143 | 0.005 | 0.080 | 0.004 | 0.112 | 0.008 |
| Mean.... | 0.138 gm. | 0.0052 gm. | 0.076 gm. | 0.0036 gm. | 0.104 gm. | 0.0048 gm. |
| $\sigma$..... | 0.0073 | | 0.0042 | | 0.0058 | |
| *C.V*..... | 5.29% | | 5.40% | | 5.57% | |
| *Em*..... | 0.00098 gm. or 0.713% | | 0.00057 gm. or 0.745% | | 0.00078 gm. or 0.75% | |

20, 23, and 25 as shown on figure 1. These figures represent points where it has been customary to take samples. From table 7, it will be seen that in the case of nitrates there are some differences between the mean of the nine and the twenty-five samples, but for most practical purposes these differences are unimportant. For instance, the mean of the nine samples for nitrates brings

TABLE 6

*Total nitrogen in soil-composite of twenty-five samples*

| SAMPLE NUMBER | PLOT 5A | | PLOT 7A | | PLOT 9A | |
|---|---|---|---|---|---|---|
| | Nitrogen content | Deviation from mean ± | Nitrogen content | Deviation from mean ± | Nitrogen content | Deviation from mean ± |
| | *gm. per 100* | *gm. per 100* | *gm. per 100* | *gm. per 100* | *gm. per 100* | *gm. per 100* |
| 1 | 0.136 | 0.001 | 0.076 | 0.001 | 0.103 | 0.000 |
| 2 | 0.137 | 0.000 | 0.075 | 0.000 | 0.103 | 0.000 |
| 3 | 0.137 | 0.000 | 0.075 | 0.000 | 0.103 | 0.000 |
| 4 | 0.137 | 0.000 | 0.075 | 0.000 | 0.104 | 0.001 |
| 5 | 0.137 | 0.000 | 0.077 | 0.002 | 0.104 | 0.001 |
| 6 | 0.137 | 0.000 | 0.075 | 0.000 | 0.103 | 0.000 |
| 7 | 0.134 | 0.003 | 0.076 | 0.001 | 0.103 | 0.000 |
| 8 | 0.138 | 0.001 | 0.071 | 0.004 | 0.103 | 0.000 |
| 9 | 0.137 | 0.000 | 0.071 | 0.004 | 0.103 | 0.000 |
| 10 | 0.137 | 0.000 | 0.076 | 0.001 | 0.103 | 0.000 |
| Mean.... | 0.137 gm. | 0.0005 gm. | 0.075 gm. | 0.0013 gm. | 0.103 gm. | 0.0002 gm. |
| $\sigma$..... | 0.0010 | | 0.00198 | | 0.00045 | |
| *C.V.*..... | 0.72% | | 2.64% | | 0.43% | |
| *Em*..... | 0.00021 gm. or 0.15% | | 0.0010 gm. or 1.0% | | 0.0001 gm. or 0.09% | |

TABLE 7

*Mean for nine uniformly distributed samples compared with twenty-five samples*

| | NITRATE NITROGEN | | |
|---|---|---|---|
| | Plot 5A | Plot 7A | Plot 9A |
| | *mgm.* | *mgm.* | *mgm.* |
| Mean of 25 samples..... | 0.49 | 0.17 | 0.32 |
| Mean of 9 samples..... | 0.39 | 0.17 | 0.29 |
| *Incubated with* $(NH_4)_2SO_4$ *and* $CaCO_3$: | | | |
| Mean of 25 samples..... | 23.8 | 4.2 | 17.4 |
| Mean of 9 samples..... | 23.4 | 4.14 | 17.0 |
| | TOTAL NITROGEN | | |
| | Plot 5A | Plot 7A | Plot 9A |
| | *per cent* | *per cent* | *per cent* |
| Mean of 25 samples..... | 0.138 | 0.076 | 0.104 |
| Mean of 9 samples..... | 0.139 | 0.077 | 0.105 |

out the expected differences between the relative productivity of the three plots just as well as the mean of the twenty-five samples. In the case of the total nitrogen determinations, the mean of the nine samples varies from the mean of the twenty-five samples by only 0.001 per cent for all three plots.

The three plots under consideration in this paper also showed a correlation between their relative fertility and the amount of nitrates and total nitrogen present. While the amount of nitrates gained or lost to a soil as determined by analytical methods might appear to be minute, such amounts have a tremendous effect upon the growth of the crop. The fact that plants use nitrates directly and draw heavily upon them at certain periods of the season, makes the determination an important one. In table 8 a comparison is made between crops yields and chemical and microbiological properties of the three plots

TABLE 8

*Comparison of crop yields as influenced by the chemical and microbiological properties of the soil*

| PLOT NUMBER | TOTAL DRY MATTER PER ACRE IN CROPS FOR 15 YEARS | NITRATE NITROGEN IN UNTREATED SOIL | NITRATE NITROGEN IN $(NH_4)_2SO_4$—TREATED SOIL | TOTAL NITROGEN CONTENT | TOTAL CARBON CONTENT | MICROÖRGANISMS PER GM. OF SOIL |
|---|---|---|---|---|---|---|
| | *lbs.* | *mgm.* | *mgm.* | *per cent* | *per cent* | *number* |
| 5A | 70,191 | 0.49 | 23.8 | 0.138 | 1.44 | 11,720,000 |
| 7A | 15,774 | 0.17 | 4.2 | 0.076 | 0.93 | 4,150,000 |
| 9A | 57,848 | 0.32 | 17.4 | 0.104 | 1.13 | 10,130,000 |

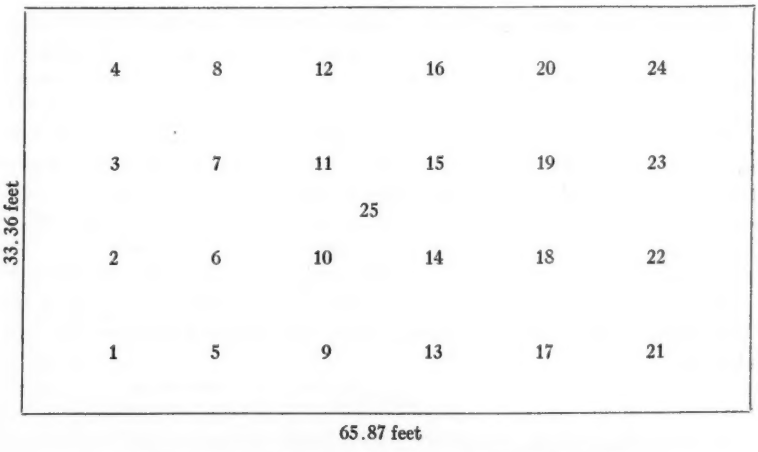


FIG. 1. DIAGRAM SHOWING LOCATION OF SAMPLING POINTS

under consideration. The numbers of microörganisms for these three plots have been recently determined by Waksman (7) from whose work the counts in table 8 have been taken. The relative fertility of these plots as measured by crop yields appears in these instances to be directly proportional to the nitrates, nitrifying power, total nitrogen and carbon content, and the number of microörganisms present. Although the nitrate content varies considerably throughout the season (2), the variations between the plots are proportionate.

## CONCLUSIONS

From this brief work, and the work of other investigators, it appears to the writer that for chemical determinations such as total nitrogen, phosphorus and potash on soil composites, where the accuracy of the analytical method is not extremely delicate and the variability of the soil is not pronounced, that nine or ten samples covering an area of one-twentieth of an acre and made into a composite would be sufficient. Duplicate or triplicate determinations on such a composite should yield results representative of the area, within the limits of the accuracy of the methods. However, when the soil constituents to be determined are changing rapidly under field conditions due to microbiological processes or to activities of plant growth, and when the method of analysis is sensitive as in the case of nitrates, a much larger number of samples must be employed to secure results that will fairly represent the area. To minimize the amount of work involved in such a process, the individual samples should be composited into several groups and these analyzed separately. The mean of such determinations would be sufficiently accurate and reliable for most practical purposes. Of course, if the coefficient of variability for a particular area has already been determined, a much smaller number of samples may be used, since the limits of variation for the area are known.

Whether a very large or relatively small number of borings should be taken in the sampling of a particular area, depends in the first place upon what the experimental data is intended to show. If comparisons are to be made between systems that have wide differences, and it is the purpose to show such differences in an approximate manner, then the number of samples need not be so great. Thus, for instance, soils from plots under different nitrogenous fertilizer treatments, would vary widely in their nitrate content and to some extent in total nitrogen. This has been shown in the work of Blair and Prince (2) on the plots at the New Jersey Experiment Station. However, where the differences between the systems under comparison are small the chance of variation between the samples within one system will be as great as the variability of the systems compared, unless the analytical methods are very delicate.

## SUMMARY

1. Variability in soils due to method of sampling was studied using nitrate and total nitrogen determinations as criteria.

2. Soil from three plots that receive different fertilizer treatment was used and twenty-five samples from each plot were taken covering an area one-twentieth of an acre in size.

3. The determinations made were nitrates on the original soil, nitrates on samples incubated with ammonium sulfate, and total nitrogen.

4. In the interpretation of the results, statistical methods were applied.

5. The crop yields from the three plots were compared and correlated with the amount of nitrate, total nitrogen content, number of microörganisms, and nitrifying power of the soils.

6. The coefficient of variability on all three plots was very great as regards nitrates and nitrification studies, ranging between 13 and 42 per cent. The probable errors of the mean in these cases were also high, ranging from 2 to 5 per cent.

7. There was a tendency for samples of soil low in nitrate also to be low in nitrifying power. There was a similar correlation in samples high in nitrate.

8. The mean of nine composite samples covering one-twentieth of an acre was in quite close agreement with the mean of twenty-five individual samples covering the same area.

9. In the case of the total nitrogen determinations, the coefficient of variability was relatively low, being about 5.5 per cent for each plot. The probable error of the mean in each case was about 0.7 per cent. Composite samples showed no variation from the mean of a large number of individual samples. Evidently, the method for determining total nitrogen is not sensitive enough to require more than one well composited sample of soil for an area one-twentieth of an acre.

10. In order to determine how many samples of soil should be taken from a given area, three questions must first be considered: (a) Is the experimental data to show large, or small differences between the systems involved? (b) How accurate are the analytical methods? (c) Are the particular substances to be measured influenced appreciably by microbiological activities and by assimilative processes of plants?

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# COMPARISON OF "ACTIVE" ALUMINUM AND HYDROGEN-ION CONCENTRATIONS OF WIDELY SEPARATED ACID SOILS[1]

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In a previous article (1), a method for the determination of "active" aluminum in soils was proposed, together with reasons for its adoption. The fact that soluble aluminum salts in acid soils are often largely responsible for decreased crop yields has been shown recently by several investigators[2] and the desirability of ascertaining roughly the extent of geographical distribution of such active soil aluminum, as well as whether or not a correlation exists between it and soil reaction, irrespective of locality, is apparent.

A large number of active aluminum and hydrogen-ion determinations have been made on the differently treated plot soils of this station, and in the paper above cited, a fairly close correlation was shown to exist between the two; i.e., the more acid soils carried the larger amounts of soluble aluminum. Some time ago, in an endeavor to extend our knowledge along this line, the writer secured through the courtesy of a number of experiment station workers, about twenty-five samples of representative acid, mineral soils from different sections of the United States and Hawaii. Active aluminum (soluble in 0.5*N* acetic acid), hydrogen-ion concentration (electrometric method), and certain other characters were determined. The laboratory numbers, the localities from whence the samples were taken and brief descriptions of each follow:

1. Rhode Island. Miami silt loam.[3] This soil came from plot 84 of the permanent field experiments of the Rhode Island Agricultural Experiment Station, is of old glacial origin, and is typical of the acid, granitic soils of the state. So far as is known, it has never received either fertilizer or lime.

2. Maine. This soil has not been mapped by the Bureau of Soils. It was taken from the Highmoor Farm of the Maine Agricultural Experiment Station, located at Monmouth

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[1] Contribution 299 from the Agricultural Experiment Station of the Rhode Island State College, at Kingston.

[2] See bibliographies appended to the papers referred to in (1).

[3] Most of the soils used have been mapped, mechanically analyzed and named by the Federal Bureau of Soils. Descriptions may be found in their "Field Operations," 1899 to date.

(Kennebec County). It is a reddish-yellow silt loam, carrying considerable fine sand, and is underlaid by a compact yellow subsoil. Tile drainage is found to be decidedly beneficial in certain localities, especially for orchard work. It is of early glacial origin.

3. Pennsylvania. Volusa silt loam. This sample came from the Bradford County Experimental Plots of the Pennsylvania State Agricultural Experiment Station. The Volusa soils are found in the northern part of Pennsylvania and in New York State. They are normally very acid and poorly drained and represent the only glaciated soils (later Wisconsin drift) to be found in Pennsylvania. They owe their origin to the glaciation of the underlying country rock, with the addition of some foreign material. No lime had ever been applied to the area from which this sample was drawn.

4. Pennsylvania. DeKalb silt loam. This soil came from a check plot of the Snow Shoe Experimental Fields of the Pennsylvania State Agricultural Experiment Station, located in

TABLE 1

*"Active" aluminum, hydrogen-ion concentration and other data on soils from different parts of the United States*

| SOIL NUMBER | LOCATION | SOIL TYPE | REACTION | ACTIVE $Al_2O_3$ IN DRY SOIL | LOSS ON IGNITION | NITROGEN | SILT* | CLAY* |
|---|---|---|---|---|---|---|---|---|
| | | | *pH* | *p.p.m.* | *per cent* | *per cent* | *per cent* | *per cent* |
| 1 | R. I. | Miami silt loam | 4.47 | 819 | 5.7 | 0.137 | 65 | 15 |
| 2 | Me. | Reddish-yellow silt loam | 4.90 | 662 | 16.6 | 0.208 | 50 | 18 |
| 3 | Pa. | Volusa silt loam | 4.45 | 270 | 11.4 | 0.264 | 61 | 23 |
| 4 | Pa. | DeKalb silt loam | 4.17 | 300 | 9.2 | 0.132 | 56 | 19 |
| 5 | Pa. | Hagerstown clay loam | 4.17 | 294 | 7.4 | 0.155 | 59 | 30 |
| 6 | S. Car. | Portsmouth sandy loam | 5.77 | 42 | 10.6 | 0.201 | 8 | 5 |
| 7 | S. Car. | Greenville clay loam | 5.70 | 72 | 4.2 | 0.041 | 14 | 33 |
| 8 | Ky. | Decatur silt loam | 4.50 | 128 | 6.3 | 0.126 | 63 | 20 |
| 9 | Ky. | Memphis silt loam | 4.72 | 44 | 4.9 | 0.087 | 83 | 9 |
| 10 | Ind. | Wakasha silt loam | 4.90 | 63 | 9.7 | 0.236 | 52 | 14 |
| 11 | Ind. | Clermont silt loam | 5.15 | 48 | 4.3 | 0.080 | 53 | 27 |
| 12 | Ind. | Scottsburg silt loam | 5.22 | 50 | 4.4 | 0.093 | 62 | 10 |
| 13 | Ill. | Upland prairie brown silt loam | 5.58 | 19 | 11.1 | 0.268 | 70 | 18 |
| 14 | Iowa | Carrington loam | 5.20 | Trace | 11.4 | 0.252 | 43 | 22 |
| 15 | N. D. | Fargo clay | 5.66 | None | 24.3 | 0.425 | 38 | 46 |
| 16 | Tex. | Lufkin fine sandy loam | 5.31 | None | 8.8 | 0.118 | 16 | 7 |
| 17 | Tex. | Subsoil of No. 16 | 5.30 | Trace | 4.2 | 0.077 | 15 | 41 |
| 18 | Ore. | Melbourne silty clay loam | 4.50 | 890 | 16.6 | 0.285 | 42 | 43 |
| 19 | Ore. | Medium sandy loam | 4.63 | 657 | 26.6 | 0.397 | | |
| 20 | Cal. | "No. 13" fine sandy loam | 4.00 | 53 | 3.3 | 0.062 | | |
| 21 | Cal. | "No. 23" fine sandy loam | 4.63 | 60 | 3.5 | 0.072 | | |
| 22† | Hawaii | Hawaii brown clay loam. † | 5.31 | 1820 | 45.4 | 0.699 | | |
| 23 | Hawaii | Oahu red clay loam | 4.65 | 537 | 25.7 | 0.349 | | |
| 24 | Hawaii | Kauai black clay loam | 4.60 | 660 | 25.6 | 0.395 | | |

* U. S. Bureau of Soils Reports. Percentages given are taken from analyses of soils from the state and usually from the county from which the above samples were drawn.

† This soil has been omitted from all averages, due to its extremely high content of soluble aluminum, organic matter and nitrogen.

Centre County, and represents forty-three per cent of the soil area of the state. It is a residual soil, occurring only in non-glaciated sections. While often acid and in a depleted condition, it responds well to fertilizers and lime and may often be profitably reclaimed.

5. Pennsylvania. Hagerstown clay loam. This sample was taken from plot 32 of the old fertilizer experiments at the State College (Centre County) Pennsylvania, and has been treated for forty years with ammonium sulfate, acid phosphate and muriate of potash. It occurs in a non-glacial area and was originally derived from limestone.

6. South Carolina. Portsmouth sandy loam. This sample was drawn near Georgetown (Georgetown County) and represents a coastal plain type of very poor, acid soil. It is low-lying, flat land of little agricultural value except for pasturage.

7. South Carolina. Greenville clay loam. This soil is also a coastal plain type coming from Trenton (Edgefield County).

8. Kentucky. Decatur silt loam. This sample came from the Russellville Experiment Field, Logan County, and is typical of considerable areas in central Kentucky. It was drawn from a non-glaciated section and is of limestone origin.

9. Kentucky. Memphis silt loam. This soil is from the Mayfield Experiment Field, located in Graves County, and is of loessial origin. It is fairly representative of western Kentucky and areas along the Mississippi River.

10. Indiana. Wakasha silt loam. This sample of soil was drawn from the Indiana Agricultural Experiment Station farm at West Lafayette (Tippecanoe County) Indiana. This type rests upon the late Wisconsin drift, and consists of glacial material, reworked and laid down by water. It does not respond to lime treatments.

11. Indiana. Clermont silt loam. This sample was taken from an untreated plot of the Jennings County Experiment Field of the Indiana Agricultural Experiment Station, located near Vernon. This is a very light colored soil (nearly white) and is of glacial origin.

12. Indiana. Scottsburg silt loam. This soil came from the check plot of the Scottsburg Experiment Field (Scott County) of the Indiana Agricultural Experiment Station. As it occurs within the Illinoisian drift area, a considerable part of the material from which this soil has been formed was deposited through glacial action. This soil responds well to lime and soluble phosphates.

13. Illinois. Upland prairie brown silt loam (2). This sample was secured from the farm of D. Smith, about three miles east of Urbana (Champaign County), Illinois, and is a typical, rich corn-belt soil. So far as is known, it has not received lime or commercial fertilizers of any kind. It is composed of fine material of loessial character from the Champaign till sheet of the early Wisconsin glaciation.

14. Iowa. Carrington loam. This soil was drawn from the experiment station farm of the Iowa Agricultural Experiment Station at Ames (Story County). It is a mellow, black soil, usually of high productivity. The material was originally formed by glacial action (Kansan drift).

15. North Dakota. Fargo clay. This sample was drawn from the old wheat plot (plot 2 in rotation series 1) of the North Dakota Agricultural Experiment Station, Agricultural College (Cass County), North Dakota. This soil has now grown forty consecutive crops of wheat.

16. Texas. Lufkin fine sandy loam. This soil, together with no. 17, which is the subsoil, was taken from the farm of the Texas Agricultural Experiment Station, College Station (Brazos County), and is the most widely distributed type of this series, occupying large areas in this section of Texas. It is flat or slightly rolling and is derived largely from grayish or drab clays and grayish, argillaceous sands. Unaltered clays and sands usually appear at about 5 feet.

17. Texas. Subsoil of no. 16.

18. Oregon. Melbourne silty clay loam. This sample was taken two miles west of Alpine in Benton County. It represents a reddish soil which prevails in comparatively large areas. It responds to lime applications.

19. Oregon. This soil has not been surveyed by the Federal Bureau of Soils but is classified by the Agronomy Department of the Oregon Agricultural College as a medium sandy loam. It was obtained a few miles north of Nahalem in Clatsop County.

20. California. "Soil no. 13." This is a fine sandy loam from the coast of Mendocino County, and is very light gray in color. It is not yet mapped by the Federal Bureau of Soils. It is probably of volcanic origin.

21. California. "Soil no. 23." This sample came from near Santa Rosa, Sonoma County, and is much improved by lime. It has not been mapped.

22. Hawaiian Islands, Hawaii. This soil came from one of the upper fields of the Honokaa Sugar Company (section 37A field 5) which is located on the windward side of the Island of Hawaii in the very heavy rainfall belt. This soil is subjected to excessive leaching. It is a dark brown clay loam. All of the Hawaiian soils here discussed are derived entirely from volcanic rock of fairly recent geologic date, and would be classed as laterites.

23. Hawaiian Islands. Oahu. This sample was drawn in the Kailua District on the Island of Oahu, from the pineapple fields of Libby, McNeil & Libby (Field no. 18). It comes from an elevation of approximately 400 feet and is in a section of fairly heavy rainfall. The soil is a brick-red clay loam, and carries large quantities of the oxides of iron, aluminum and some manganese.

24. Hawaiian Islands. Kauai. This soil came from one of the upper fields of the Koloa Sugar Company (section 6, field 54) on the Island of Kauai. The rainfall is fairly heavy. It is a black clay loam soil well supplied with organic matter.

A perusal of table 1 brings out several interesting points. At first it appears that no relationship whatever exists between the hydrogen-ion concentrations of soils of different types and the amounts of active aluminum contained therein. For instance, the most acid soil reported, no. 20 from California, with a reaction of pH 4.00, yields but 53 p.p.m. of alumina soluble in 0.5 *N* acetic acid, while the least acid one, no. 6 from South Carolina, with a reaction of pH 5.77, gives an amount of similar magnitude. As a rule, however, the soils whose pH values are less than 5; (i.e., the more acid soils,) carry much larger amounts of active aluminum than do those whose pH values are greater than pH 5. The average for the former group is 388 p.p.m. of alumina, while for the latter it is but 26 p.p.m. Thus while the acidities of the different soils may not always be directly correlated with the amounts of active aluminum found, the group averages are so correlated, as are the individual soils of varying reaction *within the same soil type.* Here a fairly close relationship between the two exists unless unusually large applications of acid phosphate or lime have been recently made. This fact has been indicated in my first paper (1) and is still more definitely shown by unpublished data soon to appear.

Another factor which tends to interfere with such a correlation between soils of different types as well as between those of the same type is organic matter. In an endeavor to shed some light on the effect of organic matter on aluminum solubility in acid soils, "loss on ignition" determinations were made. It is well understood, of course, that this loss in weight would include water of hydration or combination and possibly ammonium salts (no carbonates could exist in these acid soils) besides organic matter, but the latter would most certainly account for by far the larger portion of the losses re-

corded. There appears to be no definite correlation between the percentages of organic matter in the soils and the amounts of active aluminum, although they often vary in the same direction; i.e., the larger the loss on ignition, the greater the amounts of soluble aluminum. Just the reverse of the above finding has been shown to obtain in soils of the *same type* (1). Here large applications of organic matter appeared to decrease the amounts of readily soluble aluminum, although it should be borne in mind that the latter materials were barnyard or green manures of a readily decomposable nature, whereas in the soils reported in table 1, much of the organic matter was undoubtedly old, difficultly decomposable and but slightly soluble. As would be anticipated, the percentages of nitrogen in these soils vary with the organic matter present.

It has long been recognized that the soil materials dissolved in the soil solution, or readily soluble in an excess of any solvent, are derived almost exclusively from the finer soil separates; i.e., the silt and the clay. As clay is largely made up of hydrated aluminum silicates, it seemed of importance to ascertain whether any correlation existed between the readily soluble aluminum of acid soils and the percentages of their finer mechanical fractions. The last two columns of table 1 show the approximate percentages of silt and clay in a majority of the soils under discussion. No relationship is evident between either the hydrogen-ion concentrations or the "active" aluminum contents and the percentages of these mechanical fractions.

A careful perusal of the rainfall data for the sections from which these samples came was made by the writer where possible and it appears that in a great majority of cases, the heavier the rainfall, the larger were the amounts of active aluminum found.

The factor chiefly responsible for the presence of readily soluble aluminum in acid soils is doubtless to be found in soil genesis. Unfortunately, quantitative mineralogical analyses have not been made on the soil samples under discussion. With the exception of the laterites from the Hawaiian Islands, the glacial soils carry much larger amounts of soluble alumina than do those from the unglaciated regions, the older glaciations exceeding the more recent in this respect. This latter fact is in agreement with Slipher (3) who states, "The older the glacial age of soils, the higher the response to lime treatments . . . . The oldest glaciation has furnished the largest crop response. With the exception of the Iowan, the apparent need for lime becomes progressively less with youth."

An important fact indicated by the data here presented is that the degree or amount of soil acidity (either hydrogen-ion concentration or "lime requirement") does not necessarily indicate the amounts of active aluminum which may be present, which may explain why certain acid soils respond but feebly to lime applications. Data to be published elsewhere will show that lime unaccompanied by soluble phosphate is occasionally required in large quantities to depress adequately aluminum solubility and hence toxicity, in these cases. The idea must not be gained that ordinary lime applications are gener-

ally futile in rendering active aluminum innocuous where the latter is present in limited amounts and where the physical condition of the soil is good, but even here soluble phosphates as well as lime are advocated for quick action.

## SUMMARY

About twenty-five samples of representative acid, mineral soils were secured from as many widely separated sections of the United States, including the Territory of Hawaii. "Active" aluminum determinations by a method recently proposed by the writer (1), and hydrogen-ion determinations by the electrometric method were made, as were also "loss on ignition" and total nitrogen determinations. From these data the following conclusions seem warranted.

Considered from the point of view of a mass average, a direct correlation exists between hydrogen-ion concentration and active aluminum in acid soils, although there are several individual exceptions. The more acid soils (pH 4 to 5) averaged 388 p.p.m. of active alumina while the less acid group (pH 5 to 5.8) averaged but 26 p.p.m. (dry soil basis). The exceptions are of interest in that high active aluminum and low acidity may at times explain a lack of response to liming, for we have data which show that both lime and soluble phosphate applications are needed to depress greatly the activity of aluminum in certain acid soils.

No definite correlation was noted between "active" aluminum and the percentages of either organic matter, nitrogen, clay or silt, although often the soils rich in organic materials (and also in nitrogen) yielded the larger amounts of soluble aluminum.

A casual relationship appeared to exist between rainfall (and hence leaching) and "active" aluminum.

Soil genesis is probably largely responsible for the differences recorded in the data here presented. With the exception of the Hawaiian laterites, the glaciated soils carried much larger amounts of active aluminum than do the others, the older exceeding the more recent in this respect.

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# THE INFLUENCE OF SOLUTION VOLUME UPON PLANT GROWTH IN RELATION TO REACTION CHANGE AND IRON AVAILABILITY IN CULTURE SOLUTIONS[1]

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## INTRODUCTION

The nature and the rates of plant growth in culture solutions are determined by many factors. The relation of some of these factors to plant development is quite intricate and not at all well understood while that of others appears at first so simple as not to require much consideration. One of these apparently simple factors involved in all solution culture studies, that of the relation of solution volume or size of the culture vessel to the various types of plant activity, has, perhaps, not received the attention which its importance merits.

In some preliminary experiments dealing with the rate of change of hydrogen-ion concentration brought about by the action of growing wheat plants in two different types of culture solutions and the effect of this change upon iron availability (1) it was found that the solution volume had a marked influence not only upon the character and rates of growth directly but also upon the reaction change produced in the culture solutions and upon the manner in which the plants responded towards different compounds used as sources of iron for the plants. The phenomena observed in connection with this preliminary study appeared to be of sufficient importance to warrant further investigation.

The experiments reported in this paper were undertaken primarily for the purpose of investigating the effect of different solution volumes upon the growth rates and development of young wheat plants in two types of culture solutions. A study was also made of the hydrogen-ion concentration changes brought about by the action of the plants in the different solution volumes and of the influence of these changes upon the availability of iron added to the solutions in a soluble and an insoluble form.

## METHODS OF PROCEDURE

The culture solutions used in these experiments were Tottingham's (8) four-salt solution $T_1R_1C_3$ and a modification of this solution used by Jones

[1] Paper No. 128 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Plant Physiology.

and Shive (5). This modification consisted in substituting ammonium sulfate for the potassium nitrate of the Tottingham solution in equivalent osmotic concentrations. Baker's analyzed salts were used in the preparation of half molecular stock solutions from which the culture solutions were prepared. These were made up to have a calculated total osmotic concentration value of one atmosphere. The proportions of the salts in volume-molecular partial concentrations are given in table 1.

Iron was added to the culture solutions in the forms of ferric phosphate and ferrous sulfate. The ferric phosphate was prepared as described by Jones and Shive (4) while the ferrous sulfate was used in aqueous solution always freshly prepared just before being added to the culture solutions.

The culture vessels consisted of wide-mouth bottles having capacities of 250 500, 1000, and 2000 cc. Wheat of the "Marquis" variety was germinated on a germinating net (7) and uniform seedlings were carefully selected, mounted

TABLE 1

*Volume-molecular partial concentrations of the salts in the two types of culture solutions used*

| TYPE OF SOLUTION | VOLUME-MOLECULAR PARTIAL CONCENTRATIONS | | | | |
|---|---|---|---|---|---|
| | $KNO_3$ | $Ca(NO_3)$ | $MgSO_4$ | $KH_2PO_4$ | $(NH_4)_2SO_4$ |
| Tottingham $T_1R_1C_3$..... | 0.0020 | 0.00438 | 0.01185 | 0.00211 | |
| Modified Tottingham $T_1R_1C_3$..... | | 0.00438 | 0.01185 | 0.00211 | 0.0014 |

in the double-piece paraffined cork stopper devised by Tottingham (8) and were then transferred to the culture solutions.

The culture solutions were renewed every three and one-half to four days. Each period between two successive solution changes will be designated as a growth interval. After each solution change, the old solutions were made up to their original volumes with distilled water and samples removed for the determination of hydrogen-ion concentrations which were recorded in terms of pH values. These determinations were made colorimetrically by the use of the double-tube standards of Gillespie (3), the indicators recommended by Clark (2), and with the apparatus devised by Van Alstine (9).

Three series of cultures were conducted in each of which wheat plants were grown in both the Tottingham solution $T_1R_1C_3$ and in the ammonium sulfate modification of this solution in the four different solution volumes (250, 500 1000, and 2000 cc.) and the cultures were duplicated. The respective series are further described in connection with the presentation of the experimental data.

## EXPERIMENTAL RESULTS

### *Series 1*

In the cultures comprised in this series two wheat plants per culture were grown in the different volumes of the two types of culture solutions described.

The cultures were begun on March 4 and grown for a period of five weeks. Iron for the plants of this series was supplied in the form of ferrous sulfate in quantities of 1 mgm. of iron per liter in the Tottingham solution and 0.05 mgm. in the ammonium sulfate modification of this solution. These amounts were found by Jones and Shive (5) to give satisfactory results in the respective solutions during the early stages of the growth of wheat.

At the end of the growth period of five weeks the plants were harvested and the dry weights of tops and roots determined separately. The average dry weights of tops and of roots per plant and the average total dry weights per plant together with the hydrogen-ion concentrations of the solutions determined at the end of each growth interval are given in table 2.

TABLE 2

*Dry weights of plants and pH values of solutions at the end of each growth interval*

| SOLUTION VOLUME | AVERAGE DRY WEIGHT PER PLANT | | | REACTIONS OF SOLUTIONS AT END OF INTERVALS* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tops | Roots | Total | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| *cc.* | *gm.* | *gm.* | *gm.* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* |
| *Tottingham's solution $T_1R_1C_2$, 1.0 mgm. of iron ($FeSO_4$) per liter** | | | | | | | | | | | | | |
| 2000 | 1.5998 | 0.3027 | 1.9025 | 4.9 | 4.9 | 5.3 | 5.3 | 5.9 | 6.0 | 6.2 | 6.4 | 6.5 | 6.2 |
| 1000 | 1.7757 | 0.3197 | 2.0954 | 4.9 | 5.3 | 5.7 | 5.7 | 6.3 | 6.2 | 6.6 | 6.7 | 6.9 | 7.1 |
| 500 | 1.7435 | 0.3489 | 2.0927 | 5.5 | 5.5 | 5.8 | 6.2 | 6.7 | 6.7 | 7.0 | 7.3 | 7.5 | 7.3 |
| 250 | 1.1422 | 0.2835 | 1.4257 | 5.6 | 5.8 | 6.0 | 6.4 | 7.0 | 7.0 | 7.2 | 7.2 | 7.2 | 7.8 |
| *Modified Tottingham's solution $T_1R_1C_2$, 0.05 mgm. of iron ($FeSO_4$) per liter** | | | | | | | | | | | | | |
| 2000 | 2.5904 | 0.5014 | 3.0918 | 4.5 | 4.7 | 4.7 | 4.7 | 4.6 | 4.5 | 4.6 | 4.6 | 4.5 | 6.5 |
| 1000 | 2.3314 | 0.3832 | 2.7146 | 4.5 | 4.6 | 4.4 | 4.6 | 4.5 | 4.4 | 4.5 | 4.5 | 4.3 | 6.7 |
| 500 | 1.6885 | 0.3083 | 1.9968 | 4.5 | 4.4 | 4.3 | 4.4 | 4.4 | 4.1 | 4.5 | 5.7 | 5.0 | 7.2 |
| 250 | 1.1693 | 0.2684 | 1.4376 | 5.9 | 4.5 | 4.1 | 4.3 | 4.4 | 4.3 | 5.9 | 7.0 | 6.9 | 7.4 |

* Initial pH value of both types of solution approximately 4.7.

It will be observed from the data of table 2, that the different volumes of the Tottingham solution, except the smallest, (250 cc.), produced yields which are nearly equal in numerical value. In the 250-cc. cultures, however, growth was considerably depressed. The average dry weights of tops and of roots produced by the different volumes of the modified solution containing ammonium sulfate, on the other hand, are highest for the 2000-cc. cultures and are progressively lower for the cultures with smaller solution volumes, the lowest yields being obtained from the 250-cc. cultures. It is interesting to note also that the solution containing ammonium sulfate proved to be a better medium than the unmodified Tottingham solution for the growth of these plants in the larger volumes while in the smaller volumes (250 and 500 cc.) the two types of solutions produced practically the same yields of both tops and roots.

The pH values of these solutions determined at the end of the growth intervals are presented graphically in figure 1. The upper set of four graphs in this figure represents the pH values for the four different volumes of the Tottingham solution after contact with the roots of the growing plants, while the lower set represents in a like manner the pH values of the four different volumes of the modified Tottingham solution containing ammonium sulfate.

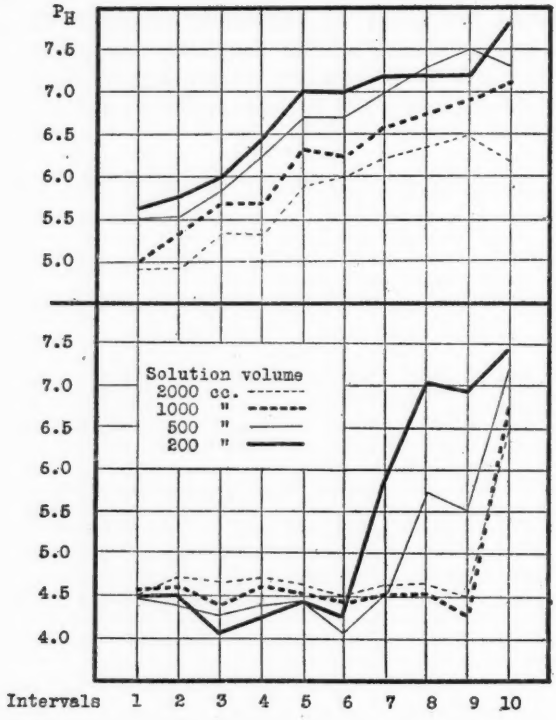


FIG. 1. GRAPHS OF pH VALUES OF CULTURE SOLUTIONS AFTER CONTACT WITH PLANT ROOTS DURING THE VARIOUS GROWTH INTERVALS

Upper graphs, Tottingham's solution $T_1R_1C_3$; lower graphs, modified solution containing ammonium sulfate.

The graphs of pH values of the Tottingham solution in this series of cultures show a gradual decrease in hydrogen-ion concentrations at the end of the growth intervals, as the plants become older, in each of the four different volumes of solution. However, at the end of any interval the greatest reaction change is always shown for the smallest volume and the least reaction change for the greatest volume. This indicates that while the rates of reac-

tion change increase with the age of the plants in each volume of solution the pH values at any time during the growth period are by no means independent of the volume of the solution in which the plants are growing.

With respect to change of reaction, results quite different from those shown for the Tottingham solution were obtained with the modified Tottingham solution containing ammonium sulfate as a comparison of the upper with the lower set of graphs in figure 1 clearly demonstrates. A slight increase in hydrogen-ion concentration over the initial concentration is observed during the first six growth intervals in each of the four different volumes of solution, the magnitude of reaction change nearly always being greatest in the smallest volume and least in the largest volume of solution. During the seventh interval, however, there was a marked decrease in the hydrogen-ion concentrations of the solutions of the 250-cc. and the 500-cc. cultures. This reversal in the direction of reaction change is shown in the abrupt rise in the graphs representing the pH values of the solutions of these two cultures in the seventh interval. At the end of the ninth interval a similar break is observed in the two graphs representing the pH values of the solutions of the 1000-cc. and the 2000-cc. cultures.

This reversal in the direction of reaction change shown by the graphs may indicate a very important stage in the development of the plants with respect to the requirements by the plants for certain nutritive ions the selective absorption of which has a pronounced influence upon the reaction of the nutrient solutions. Prince, Jones, and Shive (6) have suggested that in culture solutions containing ammonium sulfate and calcium nitrate such as were here used, the direction of reaction change is determined, in a large measure, by the relative rates of absorption by the plants of the $NH_4$-ions and the $NO_3$-ions (these always being absorbed at a relatively much higher rate than are the corresponding equivalent, oppositely charged $SO_4$-ions and Ca-ions, respectively) the selective absorption of the former tending to increase, and that of the latter to decrease the hydrogen-ion concentrations of the culture solutions. They state that during the early stages of growth the soybean plants used in their experiments drew heavily upon the $NH_4$-ions and less heavily upon the $NO_3$-ions but that this condition was reversed at a later stage of development and was accompanied by a reversal in the direction of reaction change. These suggestions are based on careful quantitative chemical analyses of the culture solutions after being in contact with the roots of the actively growing plants for definite time intervals. Assuming that these suggestions define in a general way the conditions which actually exist in the solutions here used with respect to the absorption by the wheat plants of the ions in question, it is at once clear from the graphs of figure 1 that the decrease in the hydrogen-ion concentration of the cultures solutions as indicated by the abrupt rise in the graphs representing the pH values may be the result of a pronounced physiological change in the plants with respect to the ammonium and nitrate requirement involving a change in the absorption rates of these ions which completely reverses the direction of reaction change of the culture solutions.

The influence of volume upon the reaction change of the culture solutions is clearly shown by the graphs. As previously indicated, the magnitude of reaction change always varies in the inverse order of the volumes of the culture solutions. Since any alteration in the proportions of the ions in the culture solutions brought about by selective absorption, must take place much more slowly in the larger volumes than in the smaller ones, any pronounced reaction change resulting from such absorption must, of course, be correspondingly delayed in the larger solution-volumes. It is thus observed that the marked reaction change of the solutions of the 250-cc. and the 500-cc. cultures comes at the end of the sixth interval and that of the solutions of the 1000-cc. and the 2000-cc. cultures at the end of the ninth interval as is indicated by the graphs of pH values.

## *Series 2*

As previously stated, series 2 was like series 1 except that iron in the form of ferric phosphate was added to the solutions in quantities of 5.0 mgm. per

TABLE 3

*Dry weights of plants and pH values of solutions at the end of each growth interval*

| SOLUTION VOLUME | AVERAGE DRY WEIGHT PER PLANT | | | REACTION OF SOLUTIONS AT END OF INTERVALS* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tops | Roots | Total | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| *cc.* | *gm.* | *gm.* | *gm.* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* |
| *Tottingham's solution $T_1R_1C_2$, 5.0 mgm. of iron ($FePO_4$) per liter** | | | | | | | | | | | | | |
| 2000 | 2.9540 | 0.5818 | 3.5358 | 4.7 | 4.7 | 5.0 | 5.2 | 5.5 | 5.7 | 6.1 | 6.2 | 6.5 | 6.4 |
| 1000 | 2.2626 | 0.4182 | 2.6808 | 4.8 | 5.0 | 5.3 | 5.4 | 6.0 | 6.0 | 6.4 | 6.6 | 6.7 | 6.7 |
| 500 | 1.7371 | 0.3810 | 2.1181 | 5.0 | 5.2 | 5.6 | 5.7 | 6.2 | 6.4 | 6.7 | 7.0 | 7.1 | 7.2 |
| 250 | 1.2365 | 0.2988 | 1.5353 | 5.3 | 5.6 | 6.0 | 6.3 | 6.8 | 7.0 | 7.1 | 7.1 | 7.1 | 7.3 |
| *Modified Tottingham's solution $T_1R_1C_3$, 5.0 mgm. of iron ($FePO_4$) per liter** | | | | | | | | | | | | | |
| 2000 | 3.5001 | 0.5329 | 4.0330 | 4.5 | 4.6 | 4.6 | 4.6 | 4.7 | 4.5 | 4.5 | 4.5 | 4.5 | 5.0 |
| 1000 | 3.0749 | 0.4856 | 3.5605 | 4.4 | 4.6 | 4.4 | 4.5 | 4.5 | 4.5 | 4.3 | 4.5 | 5.3 | 6.1 |
| 500 | 2.6768 | 0.4519 | 3.1287 | 4.7 | 4.6 | 4.4 | 4.4 | 4.4 | 4.5 | 5.0 | 5.2 | 5.9 | 7.2 |
| 250 | 2.1604 | 0.3591 | 2.5196 | 4.9 | 4.6 | 4.1 | 4.3 | 4.3 | 5.0 | 4.8 | 6.5 | 7.2 | 7.1 |

* Initial pH values of both types of solutions approximately 4.7.

liter in all the cultures. However, only one plant was grown in each culture in order to study the reaction change in the different volumes of solution when the rate of alteration in the proportions of the salt constituents due to absorption by the plants was reduced to a minimum under these cultural conditions. The cultures were duplicated and this series was conducted simultaneously with series 1. The numerical data are presented in table 3 and these correspond in every way with those given in table 2. The pH values as given in table 3 are shown graphically in figure 2. These graphs were prepared in the same manner as were those in figure 1 representing the pH values of the culture solutions used in series 1.

The relations with respect to reaction change produced in the culture solutions by the action of the growing plants as indicated by the graphs of figure 2, are in general quite the same as are those shown by the graphs of figure 1. This is clearly apparent from the close resemblence and the almost perfect agreement between the corresponding sets of graphs. A comparison of the graphs and of the data of pH values in tables 2 and 3 brings out the fact that the magnitude of reaction change produced by a single plant in the different volumes of solution is, with few exceptions, practically as great as that pro-

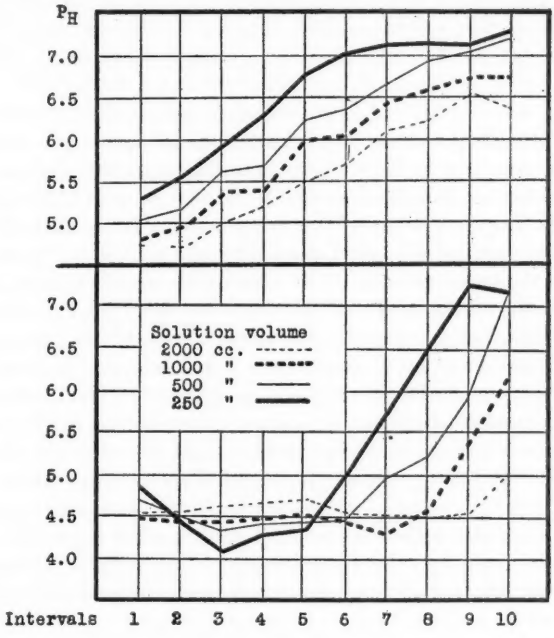


FIG. 2. GRAPHS OF pH VALUES OF CULTURE SOLUTIONS AFTER CONTACT WITH PLANT ROOTS DURING THE VARIOUS GROWTH INTERVALS

Upper graphs, Tottingham's solution $T_1R_1C_8$; lower graphs, modified solution containing ammonium sulfate.

duced by the two plants in corresponding solution-volumes. This is not surprising, however, when it is observed from a comparison of the yield data of tables 2 and 3 that the average dry weight per plant from the one-plant cultures is always much greater than that from the corresponding two-plant cultures, the greater size and vigor of the plants from the former influencing the reaction change of the culture solutions correspondingly.

The pH values of the modified Tottingham solution containing ammonium sulfate are here again slightly reduced below the initial values by the action of the plants during the first five growth intervals, after which the direction

of the reaction change is reversed but not quite so abruptly as in the corresponding solutions of the two-plant cultures of series 1. As indicated by the graphs this change in the direction of reaction from lower to higher pH values occurs first in the solutions of the 250-cc. cultures and is followed in order in the larger solution volumes occurring last in the solutions of the 2000-cc. cultures, the relative magnitude of the reaction change being determined always by the volume of the culture solution.

Examination of the dry weight data of table 3 brings out the fact that even with a single plant in each culture the growth rates are greatly retarded in the smaller solution volumes of both types of culture solutions, the average dry weight per plant from the 2000-cc. cultures being nearly double that from the 250-cc. cultures. It is also observed that the solution containing ammonium sulfate produced larger yields in each of the four volumes than did the Tottingham solution in corresponding volumes. The total average dry weight per plant in the solution containing ammonium sulfate was 14.0 per cent higher for the 2000-cc. cultures and 64.0 per cent higher for the 250-cc. cultures than were those in the corresponding volumes of the Tottingham solution, the superiority in yield being progressively less in passing from the smaller to the larger volumes. The explanation of this appears in the fact that whenever by the action of the plants the hydrogen-ion concentrations of the solutions were reduced to pH values above 6.0 the plants thereafter became chlorotic and their growth rates were checked. This condition became quite pronounced in all the plants grown in the Tottingham solution in both series 1 and 2 but did not occur at all in the plants grown in the solutions containing ammonium sulfate except to a slight degree in the plants of the 250-cc. and the 500-cc. cultures at the end of the growth period.

In both types of culture solutions there is a direct correlation shown between the reaction of the medium, determined at the end of the growth intervals, and the appearance of chlorosis in the plants thus indicating that the availability to the plants of the form of iron here used is determined by the reaction of the solution.

### *Series 3*

This series was carried out for the purpose of determining whether results somewhat like those obtained from the cultures of the preceding series could be produced at a different season of the year under similar cultural conditions and also to determine the effect of iron in the form of ferric phosphate when supplied in equal amounts to the plants in the different solution volumes of both types of culture solutions here used. The cultures of this series were conducted from September 4 to October 31. In the preceding series this form of iron was supplied in quantities of 5.0 mgm. of iron per liter of solution. Thus the total quantity of iron per culture varied from 10.0 mgm. of iron in the 2000-cc. cultures to 1.25 mgm. in the 250-cc. cultures, the total iron supplied to the plants of the different cultures being directly proportional to the

volume of the culture solutions. The present series comprised two groups of cultures. In the first group the culture solutions were exact duplicates of those of the preceding series throughout and the solutions of the second group were like those of the first except that 10.0 mgm. of iron as ferric phosphate were added to each of the four different volumes of solution so that the total iron supplied to the plants of each culture was equal throughout.

For the sake of convenience the method of supplying iron to the solutions in the first group of cultures may be designated as iron added on the "liter basis" (5.0 mgm. of iron per liter of solution), in the second group of cultures as iron supplied on the "culture basis" (10.0 mgm. of iron per culture regardless of solution-volume). In both groups three plants were grown in each culture.

In table 4 are given the average dry weights per plant and the pH values of the solutions determined at the end of the growth intervals. Since the pH values of solutions of corresponding cultures in the two groups agreed very closely, these have been averaged and the table gives only these average values for the different solution-volumes. These data apply, therefore, to the cultures in which iron was supplied on the liter basis as well as to those in which iron was supplied on the culture basis. The pH values were plotted as in the previous series to form the two sets of graphs given in figure 3.

The graphs of figure 3 show that the relations between hydrogen-ion concentration, as this is influenced by plant action, and the volume of the culture solution in which the plants were grown are quite the same as are those shown for the preceding series. It is again observed that the graphs representing the pH values of the Tottingham solutions (upper set of graphs, figure 3) lie one above the other, the upper graph representing the pH values of the solutions of the 250-cc. cultures. Below this graph, following in the order of the magnitudes of solution volume, lie the other three graphs without intersecting at any point. In the Tottingham solution the action of the plants tends always to decrease the hydrogen-ion concentration, the rate of reaction change steadily increasing with the age of the plants during the early stages of growth.

The plants in the culture solution containing ammonium sulfate, on the other hand, tend by their action to increase the hydrogen-ion concentrations of the solution above the initial concentration, during the early stages of growth. This is again followed by a reversal in the direction of reaction change which occurred first in the 250-cc. cultures during the sixth interval, in the 500-cc. cultures during the seventh interval, and in the 1000-cc. and 2000-cc. cultures during the tenth interval. This is clearly shown by the rather abrupt upward pitch in the graphs (lower set, fig. 3) of pH values for the intervals indicated, occurring in somewhat the same manner as that shown by the corresponding graphs of pH values for the culture solutions of series 1 and 2, the relative magnitudes of change in reaction being determined always by the volume of the culture solution.

Comparing now the dry weight data of the two groups of cultures of series 3 as given in table 4, it is observed that in both types of solutions the 500-cc.

TABLE 4

*Dry weights of plants and pH values of solutions at the end of each growth interval. Iron supplied in the form of ferric phosphate*

| SOLUTION VOLUME | AVERAGE DRY WEIGHTS PER PLANT | | | | | | AVERAGE REACTION OF SOLUTIONS AT END OF GROWTH INTERVALS | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group 1—Iron on liter basis (5.0 mgm. per liter). | | | Group 2—Iron on culture basis (10.0 mgm. per liter). | | | | | | | | | | | | | | | | |
| | Tops | Roots | Total | Tops | Roots | Total | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| *cc.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *gm.* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* |
| *Tottingham solution $T_1R_1C_3$* | | | | | | | | | | | | | | | | | | | | |
| 2000 | 3.4625 | 0.3758 | 3.8383 | 3.5295 | 0.3672 | 3.7005 | 5.05 | 5.35 | 5.05 | 5.25 | 5.85 | 6.10 | 5.90 | 6.35 | 6.30 | 6.45 | 6.25 | 6.30 | 6.35 | 6.75 | 6.75 |
| 1000 | 3.4600 | 0.4487 | 3.9087 | 3.4275 | 0.3915 | 3.8190 | 5.05 | 5.55 | 5.15 | 5.70 | 6.00 | 6.35 | 6.10 | 6.60 | 6.50 | 6.65 | 6.50 | 6.55 | 6.70 | 6.85 | 7.05 |
| 500 | 2.6152 | 0.3332 | 2.9483 | 3.0923 | 0.4467 | 3.5357 | 5.15 | 5.63 | 5.95 | 5.90 | 6.20 | 6.55 | 6.30 | 6.90 | 6.85 | 6.90 | 6.85 | 6.75 | 6.90 | 7.05 | 7.60 |
| 250 | 1.7852 | 0.3190 | 2.1042 | 2.5100 | 0.3225 | 2.8325 | 5.25 | 5.85 | 6.30 | 6.20 | 6.50 | 6.70 | 6.75 | 6.95 | 6.90 | 7.00 | 6.90 | 6.90 | 7.00 | | |
| *Modified Tottingham solution $T_1R_1C_3$* | | | | | | | | | | | | | | | | | | | | |
| 2000 | 5.2400 | 0.4800 | 5.7200 | 4.8782 | 0.4882 | 5.3630 | 4.83 | 5.13 | 4.86 | 4.80 | 4.70 | 4.86 | 4.83 | 5.00 | 4.90 | 5.76 | 5.30 | 5.00 | 5.70 | 5.25 | 5.95 |
| 1000 | 3.4908 | 0.3433 | 3.8341 | 3.0990 | 0.2997 | 3.3987 | 4.93 | 5.10 | 4.70 | 4.60 | 4.70 | 4.66 | 4.96 | 5.06 | 5.63 | 6.10 | 6.43 | 6.47 | 6.70 | 6.63 | 6.76 |
| 500 | 2.8088 | 0.2952 | 3.1040 | 2.8680 | 0.3483 | 3.2113 | 4.83 | 4.53 | 4.47 | 4.53 | 4.63 | 4.93 | 5.00 | 4.60 | 4.60 | 6.03 | 6.73 | 6.70 | 6.83 | 6.76 | 6.90 |
| 250 | 2.0212 | 0.3060 | 2.3272 | 2.2840 | 0.3112 | 2.5952 | 4.53 | 4.30 | 4.23 | 4.30 | 4.40 | 5.50 | 5.50 | 5.95 | 6.46 | 6.86 | 6.80 | 6.83 | 6.90 | 6.90 | 6.90 |

cultures and the 250-cc. cultures in which iron was supplied on the culture basis (10.0 mgm. of iron per culture) produced yields which were considerably higher than those from the corresponding cultures in which iron was supplied on the liter basis (5.0 mgm. of iron per liter), the ratios of the relative amounts of iron supplied by the former method to those supplied by the latter being as 4:1 and 8:1, respectively. Thus in the smaller solution volumes the application of these larger amounts of iron resulted in somewhat higher yields owing

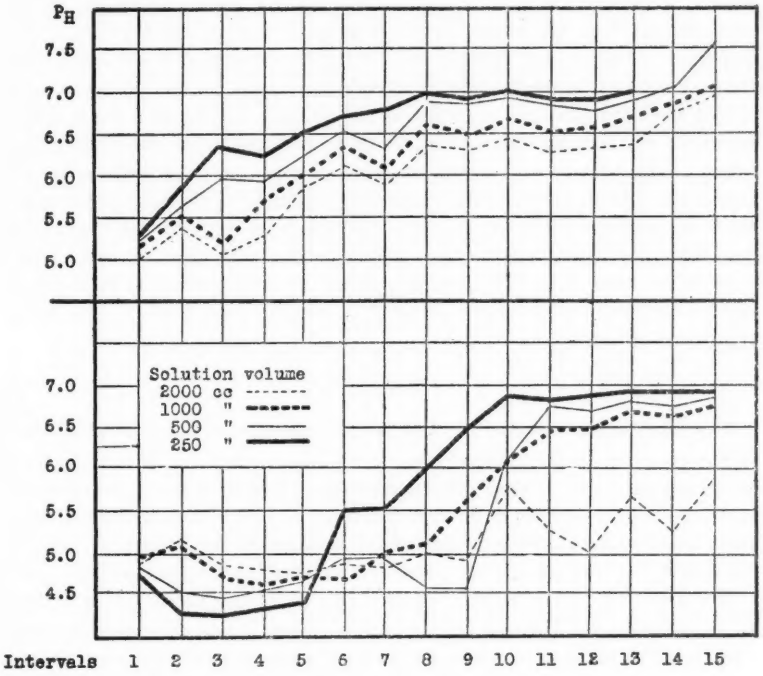


FIG. 3. GRAPHS OF pH VALUES OF CULTURE SOLUTIONS AFTER CONTACT WITH PLANT ROOTS DURING THE VARIOUS GROWTH INTERVALS

Upper graphs, Tottingham's solution $T_1R_1C_3$; lower graphs, modified solution containing ammonium sulfate.

to the fact that in the plants in these cultures chlorosis was delayed although not prevented. Chlorosis invariably occurred in the plants grown in the Tottingham solution following some time after a reaction change which brought the pH values of the solutions above 6.0 regardless of the volume of the solution in which the plants were grown or the amounts of iron added. On the other hand, the plants grown in the 2000-cc. and the 1000-cc. volumes of the solution containing ammonium sulfate were entirely free from chlorosis

appearing, however, near the end of the growth period in the plants grown in the smaller volumes of the solution to which iron was added on the liter basis.

In this series as in the previous ones there is a direct correlation between the reaction of the medium, determined at the end of the growth intervals, and the appearance of chlorosis in the plants grown in each of the different volumes of the Tottingham solution and also in the smaller volumes of the modified Tottingham solution containing ammonium sulfate.

The dry weight data of table 4 again show the retarding influence of the smaller volumes of the culture solutions upon the growth rates of the plants when the solutions are intermittently renewed at intervals of three and one-half days. This is best brought out by the dry weight data obtained from the plants grown in the modified Tottingham solution containing ammonium sulfate since the chlorotic condition of the plants grown in even the smallest volume of this solution was not sufficiently pronounced to become a disturbing factor. The average dry weight per plant of tops and total yields obtained from these cultures vary in the order of the magnitudes of the solution volumes, the lowest average yield per plant in every case being less than half that of the corresponding highest yield. Root yields, on the other hand, do not always vary in the same order as do the top yields nor is the retarding influence of small solution volumes upon the growth of roots so pronounced as it is upon the growth of tops.

## SUMMARY

The experiments described in this paper were carried out for the purpose of studying the effect of different solution volumes upon the growth of young wheat plants in two types of culture solutions. A study was also made of the hydrogen-ion concentration changes produced by the action of the plants in the different solution volumes and of the influence of these changes upon the availability of iron supplied to the plants in a soluble and an insoluble form.

The types of culture media consisted of the Tottingham solution $T_1R_1\tilde{C}_3$ at an osmotic concentration value of one atmosphere and this solution modified by substituting ammonium sulfate for the potassium nitrate in equivalent osmotic concentration. The plants were grown in volumes of 250, 500, 1000 and 2000 cc. of each type of solution.

The main results of the experiments may be summarized as follows:

1. Young wheat plants in numbers of not more than three per culture may be grown in the Tottingham solution here used in volumes of not less than 1000 cc. without undue retardation in the rates of growth, if the solutions are renewed at frequent intervals (three days or less) with a suitable form of iron supplied in the proper amounts to prevent chlorosis in the plants. On the other hand, in the modified solution containing ammonium sulfate, the growth rates of the plants varied in the order of the solution volumes from the lowest to the highest, the most vigorous growth by far being produced in the 2000-cc. cultures.

2. Hydrogen-ion concentrations are rapidly decreased by the action of the plants in the Tottingham solution in all stages of development. In the modified solution containing ammonium sulfate the plants increase the hydrogen-ion concentration of the solution slightly during the early stages of growth and decrease it during the later stages. This reversal in the direction of reaction change suggests an important physiological change in the plants with respect to the nitrate and ammonium requirements, involving a change in the absorption rates of the $NO_3$-ions and the $NH_4$-ions.

3. Other things being equal, the rates of reaction change are determined by the volume of the culture solution in which the plants are grown.

4. The availability of iron in the forms here used is determined mainly by the reaction of the culture solution as this is influenced by the action of the plants. A direct correlation is found between the decrease in hydrogen-ion concentration of the culture solutions and the appearance of chlorosis in the plants.

5. Only partial control of the hydrogen-ion concentration of the solutions in contact with the roots of the growing plants may be accomplished by the use of large volumes of solution accompanied by frequent solution renewal.

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